

RESEARCH AND DEVELOPMENT
CENTRE FOR THE STUDY OF
POLYMERIZATION



INDIAN COUNCIL OF AGRICULTURAL
RESEARCH

2001117548

Accession Number: 87-106

Issued To: *J. Charles*

**Philip Morris U.S.A.
Research Center
Richmond, Virginia**

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OCTOBER 1-31, 1987

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*N/R = No Report

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PROJECT NUMBER: 2100
PROJECT TITLE: New Product Innovations
PROJECT LEADER: C. J. Campbell
PERIOD COVERED: October, 1987

I. PROJECT HUMIDOR

- A. Objective: Develop a moisture release device for use inside a cigarette pack which will maintain the pack OV at the desired level.
- B. Status/Plans: Initial ageing test is complete on the most recent humidor pack configuration and results are favorable. A second test is now underway in an effort to duplicate the earlier results. Completion is expected in mid-November. Consumer testing of this "Freshness" concept in early 1st Quarter, 1988 is under discussion with PED.

II. PROJECT YOUTH

- A. Objective: Develop a means for maintaining fresh cigarette flavor in a hermetically sealed pack.
- B. Status/Plans: Two approaches are being pursued in an effort to reduce the off taste produced by microbial activity; elimination of oxygen inside the pack, and irradiation. Testing of oxygen reduction packets has begun, but their ability to reduce the oxygen level significantly is in doubt. An irradiation test is now being planned.

III. FLAVOR ENCAPSULATION

- A. Objective: Develop the means to encapsulate flavors for use in new or improved products.
- B. Status/Plans: At the request of FTR, a modified BLUES flavor (non water soluble) has been blended and is now in the process of being encapsulated. An objection has been raised to the use of the scented tear tape for Byzantium because the encapsulating material is urea formaldehyde. Efforts are underway to determine the quantity involved and it's potential effects.

IV. EMBOSSING CONCEPTS

- A. Objective: Explore the potential advantages of embossing cigarette paper, tipping and plug wrap for both cost savings and aesthetic appeal.
- B. Status/Plans: Various sample cigarettes with embossed tipping have been produced by machine in the Semiworks and are now being

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evaluated for dilution. A special embossing tool is being fabricated for further experimentation.

V. TAMPER EVIDENT PACK

- A. Objective: Develop potential packaging materials and processes for positively and obviously indicating an attempt to tamper with a pack.
- B. Status/Plans: A preliminary lab test of a pressurized inner bundle is in progress. An outside consulting firm, R.T. Dodge, is pursuing the development of dyes which change color to indicate a puncture through the package.

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PROJECT NUMBER: 2105
PROJECT TITLE: Filter Development
PROJECT LEADER: W. A. Nichols
PERIOD COVERED: October, 1987

I. FILTER MATERIALS, LTD.

A. Objective: Develop polypropylene tow that is equivalent to cellulose acetate.

B. Results: OC Panel testing conducted on MUL models with a CA filter vs. a FML filter with wax and triacetin indicated no significant difference in the models on all attributes tested. Sample FML filters have been made with triacetin and wax for MC Panel testing and two Mapping Tests. The smoke chemistry of these cigarettes will be analyzed.

Fiber fusion, experienced on the KDF-2 at high speed, has been reduced by the injection of compressed air at the compression tongue.

Sample mentholated cigarettes were produced with FML filters to determine menthol transfer efficiency. Previous testing had indicated a low transfer efficiency which could not be explained.

C. Plans: Various filter additives will be tested for effects on smoke chemistry. Variability in filter weight will be determined on tow produced with a new crimper design.

II. MENTHOL APPLICATION

A. Objective: Provide support for the introduction of menthol on foil.

B. Results: Various samples have been produced to support product and process introductions. Mentholated foil was produced for product testing in Spain. To qualify additional menthol suppliers, samples were produced with menthol from three suppliers. Tests are being conducted to determine the subjective and processing effects of foil oil. Processing with foil oil applied to the mentholated foil improved packer speed. Alcan foil was tested and qualified for machinability.

C. Plans: Mentholated foil samples for each of the small brands will be produced. Sample cigarettes will be produced in the factories and subjectively tested. POL testing will be scheduled for the larger menthol brands.

III. NEW FILTER CONCEPTS

A. Objective: Develop unique products using novel filters.

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B. Results:

Tube-in-Tow Filter: Tube-in-tow filters targeted for 4mg and 6 mg tar deliveries did not achieve target. Samples are being resubmitted to analytical with modified dilution.

Concentric Filters: Focus Group testing of concentric filters is in progress. Results will be available in November. Sample mentholated cigarettes were produced with blue core concentric filters for OC Panel testing. Black core concentric filters with charcoal have been received from Filtrona and will be combined in the factory.

Optional Smoking Device: Focus Group testing of menthol and regular versions of the optional smoking device is in progress. Results will be available in November.

C. Plans: Samples of the Modified Plastic Filter will be produced to the two required specifications. OC Panel testing of the blue core concentric filters will be scheduled.

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PROJECT NUMBER: 2304
PROJECT TITLE: Flavor Development - New Products
PROJECT LEADER: Barbara G. Taylor
PERIOD COVERED: October, 1987

I. STUDIO (J. Pflueger)

- A. Objective: To develop subjectively acceptable cigarettes with reduced sidestream visibility with flavor characteristics similar to Marlboro Lights.
- B. Status: Flavor-Low and Full Flavor non-menthol - The 2A blend, 8387-71A casing, and 8387-99 aftercut is the present candidate that is being tested vs. Marlboro Lights on an extended OC smoking panel.

MgO/Trim - The MgO/Trim non-menthol model was prepared for a POL test along with our regular Trim model.

II. FOAMED BOUND ROD (V. Willis)

- A. Objective: To develop a binder system that would improve coal strength, decrease loose ends, and allow for possible weight reduction without adversely affecting the taste of the cigarette.
- B. Status: A test was initiated to determine if differences exist between methods of applying the pectin - in the casing versus foamed on at maker. Subjective evaluations showed differences in the two methods of application with the method of applying the pectin to the casing as being more positive.

III. PROJECT TARGET (R. Mitchell)

- A. Objective: To develop a 16 mg tar value entry cigarette that is subjectively similar to Winston.
- B. Status: Flavor development has been initiated on Cambridge cigarettes without aftercut. A new blend has been developed by the Leaf Department, and once received, this blend will be used for further flavor work.

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PROJECT NUMBER: 2305
PROJECT TITLE: Flavor Development-Brand Modification-Menthol/Distinctive
PROJECT LEADER: H. M. Maxwell
PERIOD COVERED: October, 1987

I. ALTERNATIVE FLAVORS:

A. Objective: To identify and qualify alternative sources for several flavoring materials.

B. Status:

1. MS

POL testing on Marlboro is complete and acceptable. Testing on B&H 100 is in progress.

2. Casing Materials

A recommendation for their utilization was made.

3. Licorice

A factory run, at Stockton Street, to qualify an alternative source is being considered. Testing procedure has not yet been decided.

C. Plans:

MS-PG POL's

B&H Testing Completion November, 1987

Licorice

POL or Internal To Be Scheduled

II. PROJECT BOND

A. Objective: To develop a product with similar smoking characteristics to Prince, manufactured in Sweden. Also, to support FTR in the utilization of the Bond flavor system for an Ultra-Slim product for Sweden.

B. Status: For the product similar to Prince, cigarettes for consumer testing were produced at FTR, completing our involvement now. For the Ultra-Slim product, Semi-works models are being evaluated analytically and subjectively. Samples have been forwarded to FTR.

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C. Plans:

For Ultra-Slim (Sweden)
Evaluation of Cigarettes October, 1987

III. PROJECT MOOG

A. Objective: To develop the expertise to produce cigarettes that are subjectively equivalent to Salem, Newport and Kool.

B. Status: K-type POL's are complete. Results are being analyzed. The N-type POL cigarettes were made and rejected for analytical. They will be remade.

C. Plans:

K-type POL Results October, 1987

N-type POL Remake November, 1987

IV. PROJECT FLEX

A. Objective: For reasons of flexibility, economics and logistics, alternatives for fifteen PM flavors are being identified.

B. Status: Twelve alternative flavors have been identified. Among these are all Flex flavors used in PM domestic brands. Semi-works cigarettes, Merit and Merit menthol, are being evaluated. POL's with Merit versus Merit with Flex substituted flavors are being made.

C. Plans:

Identify 15 Alternatives November, 1987

Evaluate Semi-works
Cigarettes October, 1987

Merit POL's October, 1987

V. PROJECT GLENA. Objective:

To develop the capability to produce glycerin-free cigarettes that are subjectively equivalent to current products, for PM Germany.

B. Status:

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The second series of cigarettes was be made, using sheet materials produced in September at Park 500 and the B.L. plant. Evaluation is in progress.

C. Plans:

Series II Evaluation October, 1987

VI. MENTHOL RELEASE COMPOUND:

A. Objective: To investigate the utility of a menthol release compound for the development of a commercially acceptable product.

B. Status: To address the issues of analytical and delivery variability of menthol (ex glucosyl menthyl carbonate) analytical studies of solutions and sprayed filler are continuing cooperation with Cigarette Testing Services Division. Studies by members of Chemical Research are now indicating that menthol release of MGC is only ~20%. This may be unacceptable. Alternate venues are being considered.

C. PLANS:

Semi-works Cigarettes October, 1987
Utilizing Foam Bound Rod
Technology

VII. PROJECT LEVO:

A. Objective: To determine the subjective differences between 100% Natural and 100% Synthetic menthol and to develop flavors to make them subjectively equivalent so that manufacturing would have complete flexibility to use them at any ratio.

B. Status: A POL, Merit Menthol, was made and released for testing. Cigarettes to qualify an alternative vendor of synthetic menthol are being evaluated.

C. Plans:

POL Results December, 1987

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PROJECT NUMBER: 2306
PROJECT TITLE: Marlboro Standardization and International Support
PROJECT LEADER: J. L. Spruill
PERIOD COVERED: October, 1987

I. MARLBORO STANDARDIZATION

- A. Objective: Analytical and subjective evaluations of production Marlboro KS/LS.
- B. Status: Subjective evaluations for August 26 pick-up is complete. Standard V production runs are under analytical and subjective evaluations. Standard V King Size samples from Manufacturing, Louisville, Cabarrus and Semi-Works (DET) are being ringtipped for testing vs. Winston 85 in POL's 3558, 3557, 3556, 3560, respectively.
- C. Plans: Complete subjective and analytical analysis.

II. DOMESTIC CIGARETTE DEVELOPMENT PANEL

- A. Objective: To provide subjective direction for programs within R&D and manufacturing locations.
- B. Status: Twenty-six panels completed during reporting period.

III. PROJECT NATURAL

- A. Objective: To develop an 85mm full-flavored prototype using blend components and flavor systems which will provide a natural blended product.
- B. Status: Large-scale primary runs for Virginia, Virginia/Oriental and Blended models are scheduled for week of November 2, 1987. These models will be POL tested (3563, 3564, 3565) vs. each other. Virginia and Blended models are also scheduled for testing on POL Non-Menthol Mapping Panel (0121, 0126). Flavored models are still under evaluation.
- C. Plans: Making and evaluation of POL models. Possible testing of flavored models.

IV. FLAVOR RESOURCE DATA BANK

- A. Objective: Creation, customization and maintenance of flavor resource and data files for use by Flavor Development Division.
- B. Status: Division updated with information available in the system.

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V. INTERNATIONAL SUPPORT

Japan Smoking Panel

- A. Objective: Subjective evaluations (rod aroma and smoking characteristics) of cigarette brands in Japanese market.
- B. Status: Thirty brands received have been subjectively evaluated. Additionally, seven menthol brands have been evaluated.
- C. Plans: Issue results of subjective evaluations.

PROJECT ALPINE

- A. Objective: Develop a recessed filtered menthol product for Singapore.
- B. Status: Seven models made with recessed CA filters have been submitted for analytical. Three models were considered finished prototypes and have been evaluated. Two models selected for decision as to whether it may be tested in Hong Kong.
- C. Plans: Continue flavor development on cased prototypes.

PROJECT STARSHIP

- A. Objective: To develop a 12 mg Chesterfield for Japan.
- B. Status: Primary run for three prototypes scheduled for week of November 2, 1987. GCC L&M blend selected for models with casing and aftercut variables used. Each model will be Danchi tested vs. Kent Milds and Lucky Strike.
- C. Plans: Making and release for testing.

PROJECT MERIT/GALAXY

- A. Objective: To create and develop three new products for Japan using PM Super Lights as a base:
 - a. Merit type
 - b. Kent type
 - c. Distinctively different type.
- B. Status: Same.
- C. Plans: Same.

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PROJECT NUMBER: 2307
PROJECT TITLE: Flavor Investigation/Nonvolatile Flavor
Investigation/Processed Tobacco
PROJECT LEADER: W. R. Raymond
PERIOD COVERED: October, 1987

I. FLAVOR INVESTIGATION:

A. Objective: To provide analytical support for activities related to development and application of flavoring materials.

B. Results:

1. Casings Analysis: Comparison completed between normal phase and ligand exchange HPLC methods for polyols and sugars in casings. Both methods yield satisfactory results for general casings analysis. However, the former method appears superior for analysis of Burley spray while latter performs best for Bright casing. Both methods will be made available for Q.A. use.
2. Flavor Analytical Support: Concentration and identities of major components of five essential oils and one flavor chemical determined by GC and GC-MS. Several flavor samples analyzed for ethanol content by GC. Thirty tobacco extract and oleoresin samples analyzed for nicotine content by new rapid GC method.
3. Packaging Materials Analysis: Headspace volatiles of two new plastic boxes examined by GC for Project FOX. Polypropylene boxes confirmed to have higher volatile content than polyethylene. Headspace volatiles of UV-cured Carleton and Marlboro Lights Menthol cartons compared by GC. Both found to contain methoxyethylbenzoate and benzophenone as major UV absorbers although residual concentrations of both varied widely among cartons.

II. NONVOLATILE FLAVOR INVESTIGATION:

A. Objective: To investigate tobacco-natural, tobacco-identical and other nonvolatile fractions and constituents for potential application as cigarette flavor additives.

B. Results:

No activity this month.

III. PROCESSED TOBACCO:

A. Objective: To develop basic and applied knowledge for the purpose of improvement or selective modification of subjective properties of processed tobaccos.

B. Results:

1. Cigarettes for POL's 3548 and 3549 (dry flavor replacement in recons) shipped. Results anticipated mid-November.
2. POL's 3550-3552 (modified RLB in Marlboro) released to be shipped end of October. Process development continues to resolve insoluble solid rejection in size press. Currently evaluating particle size reduction using a homogenizer. Subjective evaluations in progress with latest pilot trial sheets.
3. Subjective evaluations in progress on post-ART stem utilization in sheet and via direct incorporation in blend. Initial results on post-ART unwashed shredded stems promising. Tests in progress to confirm earlier results at 5% replacement of ESB-2 as well as to determine any differences at 12% level in Marlboro.
4. ASTA test sheets made at FTR selected for evaluation in Spanish Marlboro Semi-Works blends at projected levels. Partial analytical data available on test sheets.

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PROJECT NUMBER: 4009
PROJECT TITLE: Development Smoke Studies
PROJECT LEADER: B. L. Goodman
PERIOD COVERED: October, 1987

I. PROJECT STUDIO

- A. Objective: Develop subjectively acceptable cigarettes with reduced sidestream visibility.

C B. Status: The 12% Mg(OH)₂ wrapper was used on a 7mg tar model with the 2A blend for an extended test on the Internal Panel. Results are being tabulated. The same model also went to the Factory Panel, where it received an acceptability score of 4.0 versus a Marlboro Lights control with a 4.2 rating. This difference was significant. The same cigarette was also made in a 100 mm version, delivering 10mg tar. The Studio Panel found the model comparable to a Marlboro Lights 100 control. A new set of control and experimental cigarettes will be made with identical appearances for testing on the Factory Panel.

The same paper, blend, and flavor system as above in a 4 mg tar construction was tested on the O/C computerized panel with a Merit Ultra Lights control. There were no significant differences in any of the attributes for the single stick evaluations. Extended smoking is to be pursued.

An Ultra Slim model at 7mg tar with the 12% Mg(OH)₂ wrapper was made with a modified blend 2A for POL testing vs. Capri. The same cigarettes were also hand packed for an extended O/C panel test versus the regular TRIM model. Sidestream visibility reductions were 20-25 % for the regular TRIM and 70 % for the Mg(OH)₂ model compared to conventional cigarettes. A menthol companion is being designed with higher levels of menthol than previous prototypes.

Subjective and analytical differences between citrate and acetate additives on Mg(OH)₂ wrapper were explored. The burn rates and CO deliveries showed some variation, but were not consistent. Ecusta maintains that it requires higher levels of citrate than acetate to achieve the same burn rate, and since the level of acetate is already at 4-5 % they have continued using acetate. There is already acetate in the base sheet due to residue from the precipitation process for making Mg(OH)₂. Subjectively there are slight differences between the two additives, primarily in terms of smoothness and impact. A majority of Studio Panel smokers preferred the acetate model.

- C. Plans: Complete Internal Panel Testing and evaluate results.

Make a Full Flavor, 60% reduced visibility model for internal testing.

Continue development of models with perforated, high Mg(OH)₂ levels and expanded tobaccos.

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II. TIPPING PAPER INVESTIGATIONS

- A. Objective: Evaluate alternate tipping papers and plug wraps for their ability to conceal the joint on cigarettes with dual black/white filters.
- B. Results: Six filter models were made with different plug wraps on the inner black section, the outer white section, and the combined filter. Each combination was made into cigarettes using five different tipping papers. The cigarettes were then tested for appearance on the O/C Panel.

Test results showed that the visibility of the joint between the two filter sections can be reduced or eliminated by changing the current Malaucene tipping to a higher opacity, 4lg sheet in combination with a higher opacity combining wrap or by changing both the plug wrap and the combining wrap to one with a higher opacity. In this test the mechanically perforated combining wrap gave a better appearance than a high opacity porous combining wrap. Just changing the tipping paper gave an improvement, although it was not as noticeable as if the combining wrap was also changed.

Coal drop-off and filter flare-up testing of the control Malaucene tipping paper showed problems with the higher opacity combining wraps, while the experimental 4lg tippings from both Ecusta and Kimberly-Clark performed well.

- C. Plans: Assess the manufacturing impact of possible improvements.

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PROJECT NUMBER: 2304, 2305, 4015
PROJECT TITLE: New Product Development
PROJECT LEADER: B. G. Taylor, H. Maxwell, and W. G. Houck
PERIOD COVERED: October, 1987

I. MARLBORO/MARLBORO LIGHTS MENTHOL

A. Objective: (1) Develop Marlboro Menthol to appeal more to Salem smokers. (2) Develop a Marlboro Lights Menthol brand extension to appeal to Salem Lights smokers.

B. Status: (1) National start-up production to begin November 16. Products to include 83 mm box Lights, 100 mm box Lights, and 85 mm Full Flavor SP. Additional testing to be done to address handling methods for menthol-on-foil (MOF) products. (POL's 2146-49)

C. Plans:

Complete latest POL Testing (1st Replicate)	11/87
Monitor Test Markets	On-going
Product Start-up	11/87
National Market	2/88

II. PM BLUES

A. Objective: To develop a nonmenthol Full Flavor distinctively flavored product using the basic PM Blues flavor system.

B. Status: First Blue II prototype currently on nonmenthol mapping panel. Second prototype under development.

C. Plans:

Review Mapping Results	11/87
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III. MARLBORO ULTRA LIGHTS

A. Objective: To develop 85/100 mm Ultra Low (6 mg) candidates for Marlboro line extensions.

B. Status: Eight POL tests currently in progress testing 6 mg - 85/100 mm products with two blend systems. Results available second week in November, 1987.

C. Plans:

Review POL Results	11/87
Test Market	1st Qtr. '88

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IV. PROJECT EXTRA

- A. Objective: The development of 4-8 mg product candidates that have the subject taste of product with twice the tar.
- B. Status: Mapping studies continue. Paper/CA candidates scheduled for POL and Mapping studies. CR chemical research and evaluation continues for possible new/enhanced flavor systems.
- C. Plans:
- | | |
|------------------------------|----------|
| Sensory mapping | On-going |
| Paper/CA Consumer Testing | 11/87 |
| Flavor Development/screening | On-going |

V. PROJECT TRIM (Ultra Slim)

- A. Objective: To develop 17 mm circumference/100 mm Ultra Slim product candidates.
- B. Status: Phase III POL Testing completed (TRIM Regular and Menthol vs. Capri) with satisfactory results. POL Testing of TRIM with and without Mg(OH)₂ paper in progress. Results available first week in December.
- Phase IV mall testing with increased menthol level and new blend scheduled for early December 87.
- C. Plans:
- | | |
|----------------------------|-------|
| Phase IV Mall Testing | 12/87 |
| POL Testing vs. Capri | 11/87 |
| Test Market Prod. Start-up | 1/88 |
| Test Market | 3/88 |

VI. PROJECT ART

- A. Objective: To investigate methods to decrease the nicotine content in tobacco. To develop full-flavored, ultra low, and light products using the low nicotine tobacco products.
- B. Status: Development continues on 6, 8, 11, and 16 mg prototypes with menthol and improved flavor system. Nicotine add-back studies planned. Blend studies in progress. 15% inclusion GCC blend in progress.
- C. Plans:
- | | |
|---|----------|
| Flavor/Prod. Development | On-going |
| Pilot Plant Material Eval. | On-going |
| Blend Evaluation (From Pilot Plant run) | 11/87 |

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PROJECT NUMBER: 4022
PROJECT TITLE: International Product Development
PROJECT LEADER: L. Mims Evans, R. E. Tinker
PERIOD COVERED: October, 1987
WRITTEN BY: A. H. Confer

I. PROJECT SPEEDBOAT (HONG KONG)

- A. Objective: Develop an American blended KS product at 9 mg. tar.
- B. Results: U.S.A. Merit (white tipped) and Kent (from the Hong Kong market) have been ring tipped and shipped to Hong Kong for consumer testing. Awaiting results.
- C. Plans: Additional prototypes are scheduled for production the week of November 2. Control is Marlboro Lights with plain white tipping; Test is Merit-type with 27 mm filter, GCC blend, and plain white tipping.

II. PROJECT ALPINE (SINGAPORE)

- A. Objective: Develop a subjectively acceptable recessed filter menthol product.
- B. Results: Seven prototypes are being screened subjectively.
- C. Plans: Recommendations will be made to Hong Kong management during the NPC meeting in November.

III. PROJECT DANNY (MALAYSIA)

- A. Objective: Increase inclusion of local tobacco in Marlboro while attempting to maintain taste character.
- B. Results: A consumer test of 35.0% local inclusion vs. 24.6% has been completed and the 35.0% is now in production.
- C. Plans: One prototype with 40% local inclusion and two prototypes with 50% will be fabricated when recon is received from Malaysia.

IV. PROJECT MLM (SPAIN)

- A. Objective: Develop Marlboro Lights Menthol Box for Spain.
- B. Results: Three bobbins of foil have been received in Richmond, mentholated at three different levels, and shipped back to Spain. Prototypes will be fabricated in Spain.
- C. Plans: A December launch is planned.

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V. PROJECT LTN (VENEZUELA)

- A. Objective:** Develop a reduced delivery product with nicotine delivery less than 1.0 mg/cigt.
- B. Results:** Prototypes with 30% ventilation have been produced and evaluated.
- C. Plans:** Prototypes with reduced ventilation (and other adjustments) will be produced and evaluated prior to the Latin America Operation Meeting in Caracas in late January.

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PROJECT NUMBER: 4024
PROJECT TITLE: Japan Product Development
PROJECT LEADER: C. B. Altizer
PERIOD COVERED: October, 1987

I. MERIT 7 mg (JAPAN)

- A. Objective: Develop a 7 mg product for Japan, with full flavor response, to achieve parity with Mild Seven smokers.
- B. Results: Results of PMI tests 1, 2, and 3 indicate that the Mount blend continued to test well. PMI and Danchi test results indicate that there are no apparent advantages to using a plastic fluted filter for this product. PMI Test IV, consisting of Merit IV (7 mm CA/14 mm CA with carbon filter, and Merit Aftercut) versus Mild Seven Lights and Caster is in progress. Results are expected by December 1.
- C. Plans: The factory trial has been tentatively scheduled for mid-November. Production startup is scheduled for early December. Product launch scheduled for March 1988.

II. MOUNT @ 11 MG vs PM LIGHTS

- A. Objective: To determine the consumer acceptability of changing the PM Lights blend to Mount blend at 11 mg tar. To test consumer preference for white versus cork tipping.
- B. Results: The Danchi Panel Test results of cork vs. white tipping on both PM Lights and Mount at 11 mg showed the PM Lights product with white tipping to be the highest rated product. In general, smokers preferred tipping paper the color of their own brand. Smokers perceived cork tipped cigarettes as being stronger and more irritating. Results of PMI testing (PM Lights vs Mount 11 mg both with white tipping) are expected mid-November.
- C. Plans: Further development plans will be based on the results of PMI testing.

III. LARK OPTIMIZATION

- A. Objective: To optimize the subjective acceptance of the Lark family while retaining the American blended cigarette character.
- B. Results: Models of optimized Lark and Lark Milds were submitted for Danchi Panel Testing. Results are expected by mid-November. Additional blend development has been done, resulting in a new blend model for another Danchi Test.
- C. Plans: Lark Milds and Full Flavor Lark prototypes with the new blend will be Danchi Panel tested mid-November. Further development work is dependent upon Danchi Test results.

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PROJECT NUMBER: 5001
PROJECT TITLE: Packaging Studies
PROJECT LEADER: H. R. Dunaway
PERIOD COVERED: October, 1987

PACKAGING STUDIES

A. Objective: Assist New Products Directorate in evaluating new packaging concepts and products, and provide technical packaging support to Manufacturing, Manufacturing Services, Engineering, Purchasing, and Quality Assurance.

B. Status/Plans:

1. Project Fox: The most recent box samples from Nypro continue to contain large amounts of hydrocarbons and prove to be subjectively unacceptable. Since these samples were made, Nypro has modified the mold to permit double gating which will allow them to lower process temperature and reduce resin degradation and odor. Confidentiality agreements are being implemented with resin and pigment suppliers so that material compositions may be divulged while attempts to reduce volatiles continue. Nypro is also investigating additional process modifications that possibly could help drive off existing volatiles. The next sample run will incorporate several non pigmented resins, and if one is found to be acceptable, pigment will be blended with the same material in order to eliminate additional volatiles.
2. Project Fresh: Sample box blanks have been ordered with new green and gold inks to reduce ink rubbing and cracking problems. Inner foil with new ink to eliminate the foil adhering to itself has been received. These materials will be subjectively evaluated provided that machine testing is favorable.
3. Cigarette Case Overwrap: The twelve week environmental test has been completed and a report is in progress. The data indicates that carton overwrap greatly enhances OV retention under hot/dry conditions, and as would be expected, foil performs somewhat better than polypropylene. Overwrapping the case, rather than individual cartons, with polypropylene provides comparable protection.
4. Scented Tear Tape: Considerable reduction in peel strength over time indicates that delamination of the tape is taking place regardless of environmental conditioning. Currently in progress is a comparison of 4mm strips stored under the same conditions, with those taken from packages, to determine the effect of the packing process itself.
5. Vaassen Foil: Six week environmental testing under Lab, Jungle, Desert, and Cold conditions showed samples from this new foil supplier to be comparable to control foil from Golden Belt.

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6. Archer Foil Laminated by Golden Belt: Environmental testing of this material, as well as subjective evaluation of cigarettes packaged with the material, show it to be comparable to Golden Belt's control foil.
7. Foil Oxidation: Six week environmental testing on packages using Reynolds foil, from a lot which showed oxidation on the bobbin while stored under summer warehouse conditions, has been completed. Results indicate that if oxidation is not visible when the foil is used to package cigarettes, it does not occur within the pack after six weeks storage under Lab, Jungle, Desert, or Cold conditions.
8. Taste-Odor-Stale Customer Complaints: Marlboro KS SP cigarettes conditioned to OV's of 8.4, 10.4, 11.8, and 14.5 (500 each) were provided to PED for evaluation on the M/C panel.

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PROJECT NUMBER: 0400
PROJECT TITLE: Low Density Rod Development
PROJECT LEADER: R. S. Mullins
PERIOD COVERED: October, 1987

LOW DENSITY ROD

- A. **Objective:** Develop a continuous process for the production of reduced density cigarettes.
- B. **Results:** Efforts this month have concentrated on starting up the Max III tipper installed on the low density rod cigarette maker. Since this tipper was obtained from the warehouse, considerable time has been required to identify and correct storage-related problems such as missing or frozen parts. In addition, several parts had to be changed to configure the tipper for production of cigarettes in the desired Marlboro Lights configuration. The softness of the low density cigarette rods also required the tipper to be adjusted to more precisely position both the filter and cigarette rods as they enter the tipper to minimize the need to "push" the rods into alignment. Thus far, the longest continuous run made on the tipper was 1/2 hour in duration during which 50 mg light cigarettes were produced at a production rate of 1000 cpm. Since the tipper now appears to be operating well mechanically, development efforts are being directed towards the tipping of cigarettes having a 25% density reduction.

The difference in firmness between conventional cigarettes containing coated (6% Genu L200) but unreactivated filler and cigarettes containing non-coated filler was found to be much greater than was predicted by the firmness model based on the CV difference of the blends. The control filler, which had a CV of 4.73 cc/g, produced a cigarette firmness of 4.1 mm. The model projected that the coated filler, which had a CV of 5.10 cc/g, would produce a cigarette firmness of 3.67 mm, an improvement of 0.43 mm. The measured firmness of the cigarettes produced with the coated filler, however, was 3.14 mm, an improvement of 0.96 mm. This result might indicate that shred stiffening is a larger contributor than previously thought to the improved firmness of bound rods produced using pectin as a binder. Additional testing is planned to determine the reason for the discrepancy.

- C. **Plans:** Undertake the on-line tipping of bound cigarettes having a 25% density reduction. Identify any problems associated with tipping low density cigarettes on-line. Evaluate the effect of cut width on the properties of cigarettes produced from the all lamina blend. Investigate the CV/firmness relationship of pectin-coated filler.

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PROJECT NUMBER: 1307
PROJECT TITLE: Reconstituted Tobacco Development
PROJECT LEADER: R. G. Uhl
PERIOD COVERED: October, 1987

I. IMPROVED SHEET PROPERTIES

A. Objective: Improve the physical characteristics and blend performance of reconstituted sheet materials.

B. Results:

1. ART Project - Spent stems (unwashed bright) from the ART pilot plant were used to replace 35% of the bright stems in pilot RL feedstock. Subjective screening of handmade 23% cigarettes showed some detectable subjective differences versus controls, particularly with 150B sheet. Machine-made Marlboro blends will be used for more extensive evaluation. Additional pilot sheets are being made from other ART stem lots (same stem type) to determine subjective consistency.

In order to determine whether there is a subjective impact of ART stems attributable to monopotassium citrate, pilot RL was made in TC, 150B and Modified 150B formulations with citrate added to the size. Citrate levels simulated replacing 1/3, 2/3 and all of the bright stems in normal RL feedstock with ART stems. Handmade cigarettes were submitted for subjective screening.

The effect of citrate on RL fiber processing was evaluated by adding monopotassium citrate to process liquors (SBW, RBW) and fiber chests. Both test and control were targeted at 12% baseweb HWS to approximate Park 500. Citrate to HWS ratios were maintained at an elevated level, equivalent to replacing all bright stems in the feedstock with ART stems. There was a slight increase in sheet adhesion to the Yankee Dryer. Sheet tensile strength was somewhat lower, but additional testing will be required to determine statistical significance. There were no measurable differences in paper machine moisture profile, fines retention or drainage rates. The system developed several leaks at threaded fittings, gaskets and pump seals. This is attributed to a cleaning action on sediments due to the slight acidity and considerable chelating properties of citrate.

None of the pilot RL sheets made using ART pilot plant spent stems or having added monopotassium citrate have shown the appearance of surface salts during sheet equilibration. This has been noted only with sheets made from the Bremen-3 stems.

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2. Humectants - The second round of POL results on humectant rearrangement blends (using sheet materials with reduced humectants) again showed equivalence to control. Plans are being formulated for a factory blend evaluation.

Glycerin-free blends utilizing production sheet materials with PG as the only humectant were completed in Semiworks. Subjective results show no significant difference versus controls.

PG/glycerin-free pilot RL sheets and RCB handsheets were made using potassium complexed propyl paraben and several levels of isosweet.

3. TTG Support - Sieve data from Semiworks garniture runs on Philippine Heiboflake sheet confirmed Semiworks primary data. Heiboflake test sheets showed no improvement over Heiboflake controls and survival significantly less than RCB. Since both Heiboflake and RCB have higher sheet strength in the machine direction, the small-scale impact tester was used to compare survivability of sheets shredded in the machine direction vs cross-machine direction. The Heiboflake materials dusted badly when shredded in the cross direction, as did the control and several tests shredded in the machine direction. Sieve profiles after impact testing showed that 3 (of 8) Heiboflake test sheets shredded in the machine direction approximated domestic RCB shredded in the cross-direction.

- C. Plans: Expedite factory trial for final approval of humectant rearrangement blends using reduced humectant sheets.

II. SUBJECTIVE MODIFICATION OF RL

- A. Objective: Improve or modify the subjective character of RL.

- B. Results:

1. POL cigarettes to evaluate dry flavor replacements in RL 150B and RCB were mailed to panelists.
2. POL cigarettes to evaluate Park 500 Modified 150B at three blend levels were mailed to panelists.
3. Additional lab testing with pilot CEL verified that the Gaulin homogenizer reduces calcium/magnesium phosphates to a size small enough to pass multiple muslin layers in a lab centrifuge, treating either ammonia-adjusted CEL plus DAP or the final Modified 150B size. Other testing indicates that the precipitate will break up even at relatively low homogenizer pressure (i.e., impact velocity). It also responded to non-impact shear treatment in a lab blender and the Cowles dissolver. Commercial in-line homogenizers are typically shear devices.

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4. Pilot plant ability to reproduce Park 500 size press nip rejection is hindered by small roll diameter, low line speed and lower size press nip pressure. Control and test (homogenized) Modified 150B sizes were nevertheless evaluated using Park 500 materials (baseweb and CEL), maximum line speed (80 fpm) and maximum nip pressure. Test size viscosity held steady while control size viscosity increased during the sizing runs. However, the lab centrifuge test showed that homogenization was only partially effective with the Park 500 CEL. Chemical analysis of the finished sheets, as well as the initial and final sizes, showed size press phosphate rejection for both the control and the homogenized test. This is being repeated to provide a direct comparison (phosphate rejection and efficacy of homogenization) of Park 500 and pilot CEL. Shearing devices will also be tested, and effort will be directed at qualifying a lab test to accurately predict nip rejection.

- C. Plans: Demonstrate precipitate size reduction as a means to eliminate nip phosphate rejection.

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PROJECT NUMBER: 1313
PROJECT TITLE: Semiworks Process Development
PROJECT LEADER: D. E. Albertson
PERIOD COVERED: October, 1987

A. Objective: Provide engineering and technical support to improve the performance and efficiency of the Semiworks operation.

B. Results:

Production of 150-lb Samples of Filler in the Semiworks (Skidmore) - A project was initiated to determine the minimum sample size that can be processed in the Semiworks primary. Emphasis of the project will center on the ADT dryer operation and control which is currently the limiting factor on running small samples. The project is expected to take 60 to 90 days and will be coordinated with the Semiworks Process Control group.

Make/Pack (Albertson) - Installation of the third Max-S tipper and Accuray unit was completed in October. The fourth and final unit is scheduled to be installed during the early part of November, completing the upgrade of the Semiworks Mk-9 complexes.

C. Plans: Transfer the technology developed for the TLA primary process to the Semiworks small scale primary. Complete the upgrade of the make/pack equipment in the Semiworks. Continue work to improve the performance of the burley P&S dryer.

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PROJECT NUMBER: 1333
PROJECT TITLE: Semiworks Process Control
PROJECT LEADER: D. A. Phan
PERIOD COVERED: October, 1987

A. **Objective:** Evaluate and revise the process control and data acquisition system to improve processing performance and production quality.

B. **Results:**

Semiworks Primary Computer Upgrade (Oliver/Sims) - Work is underway to reconfigure the backup HP computer to provide direct access to the Fisher Provox database and to eliminate the data communication link between the HP host and backup computers. This work is being conducted to improve the performance and reliability of both computer systems.

Two process control and data acquisition software packages, the Onspec from Heuristics Inc. and the Camm from Centec Corp. have been evaluated for use in the Semiworks primary. The Camm software package was selected for the application because it provided more features. This software package, run on an IBM-AT with a special interface connected to the Fisher Controls Provox highway, will provide greater flexibility in the handling of historical process data.

Integration Feeder Throughput (Phan/Sims) - Work is underway to see if improvements can be made to the feeder and weighbelt systems in the total blend area to satisfy the desired tobacco flow rate requirement of 750 lb/hr wet basis. Modifications have been made to increase the speed of all the integration feeders. Initial results indicate that all feeders can now deliver the desired flow rate of 750 lb/hr.

Adt Dryer Control Improvements (Sims) - The existing ADT dryer process control system is being reviewed to see if any improvements can be made to reduce overdrying at startup and shutdown when processing small (150 lb) samples.

C. **Plans:** Continue the work on the HP system reconfiguration and the Adt dryer process control improvement project. Complete all testing of modified integration feeders and weighbelts. Continue conducting routine QA functions.

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PROJECT NUMBER: 1503
PROJECT TITLE: Modified Smoking Materials
PROJECT LEADER: J. G. Nepomuceno
PERIOD COVERED: October, 1987

I. FOAMED FILLER BINDER

- A. Objective: To develop a process for applying a subjectively acceptable foamed binder to the tobacco filler during making to improve cigarette coal strength, reduce loose ends, increase firmness and allow for weight reductions.
- B. Results: The degraded pectin received from Grindsted, Inc. (TSP-095) was applied to Cambridge Lights cigarettes at a 1.0% addition rate using a 35% degraded pectin solution. Results to date show that this binder is matching the performance of the pectin binders developed in-house. Additional testing is in progress to establish the effects of tobacco OV adjustment on pack firmness. Cigarettes with this binder system are currently being evaluated by our internal panels. Degraded pectin binders prepared via the pilot scale continuous degradation unit were also evaluated this month on Cambridge Lights cigarettes. Results show that these binders are also matching the performance of the binders prepared via the lab scale batch process.
- C. Plans: Complete evaluation of the pectin samples from Grindsted including the subjective evaluation of these prototypes. Establish whether firmness improvements demonstrated to date warrant the initiation of mail-out testing with these prototypes.

II. ENGINEERING SUPPORT:

- A. Objective: Provide technical support to Engineering during the factory implementation of the menthol in foil program.
- B. Results: Experiments to determine the level of ethanol in the pack 12 hours after packing were conducted in support of the menthol in foil program implementation. In addition, ethanol permeability studies through the plastic bags were conducted. Results from these studies were used to track the fate of the ethanol from application to 12 hour old packed product. The ethanol was applied along with the menthol.
- C. Conclusions: Results show that a major portion of the ethanol applied to the bobbin during the menthol application step is lost at the packer. These results have been verbally communicated to Engineering and a report has been written.

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PROJECT NUMBER: 1806
PROJECT TITLE: New Tobacco Processes
PROJECT LEADER: S. R. Wagoner
PERIOD COVERED: October, 1987

I. PROJECT ART - PILOT PLANT SUPPORT

A. Objective: To provide processes for converting and casing stem materials into useable form for the ART Pilot Plant.

B. Results: Daily shipments of monopotassium citrate cased stems continued to the ART Pilot Plant. Shredded bright stems were produced on the D Pilot stem line from blended VST feedstock obtained from the Louisville ES Plant. These stems were shredded to either the standard ART size or to a finer size suitable for direct cigarette inclusion.

Since the ART Pilot Plant needs more filler drying capacity, tests were conducted drying post-ART filler in the Semiworks Hambro dryer and the larger of the two D Pilot Adt dryers. The filler for both runs contained numerous frozen clumps. While many of these filler clumps passed through the Hambro unit without breaking up, there were no clumps visible exit the Adt dryer. Thus, Development Engineering has begun work to relocate the Hambro dryer to the D Pilot stem line and the large D Pilot Adt dryer to the ART Pilot Plant.

C. Plans: Continue to produce stem products as required by Project ART. Coordinate the exchange of dryers among the ART Pilot Plant, Semiworks, and D Pilot Plant.

II. PROJECT ART - COMMERCIAL PROCESS DEVELOPMENT

A. Objective: To conduct trials providing information for development of the ART commercial process.

B. Results: Cigarettes were made utilizing post-ART stems that were of the proper size for direct blend inclusion. These were Marlboro-type blends that included 5 or 12% as-is post-ART stems, post-ART stems superheated in the 3" tower, shredded stems without K-citrate or ART treatment, and control ES. Subjective analysis is ongoing by Product Development.

Two trials were coordinated with the ART Pilot Plant in which ammonium bicarbonate was applied to Marlboro cut filler (w/o DIET and ESB) at a target cutting moisture of 19%. This was to determine whether the drying step could be eliminated after the cutter and prior to AB application in the commercial process. The trials showed this approach to be feasible, as long as the AB concentration is maintained within solubility limits.

Assistance was provided to Entomological Research in conducting a test to determine the effectiveness of the Hauni steam tunnel

(in Louisville) in killing cigarette beetles. The purpose of the test was to determine if the need for a vacuum conditioner could be eliminated, by qualifying a steam tunnel for bug kill. Preliminary results showed that the steam tunnel achieved an excellent degree of success in killing the four life stages of the beetle.

- C. **Plans:** Complete subjective analysis of the cigarettes containing post-ART shredded stems.

Conduct a test to determine the effectiveness of the steam conditioning cylinder in the Louisville ES Plant in killing cigarette beetles.

III. BINDER DEVELOPMENT

- A. **Objective:** Develop methods to produce binder systems for the foamed bound rod, bonded ends, and low density rod programs.
- B. **Results:** A sample of degraded citrus pectin, Genu X-7994, was received from Hercules. This sample was spray dried after degradation, instead of being alcohol precipitated and drum dried as with previous samples. As expected, this produced a different molecular weight distribution. Binder solutions were reformulated from this material, from Grindsted TSP-095, and from in-house batch and continuously produced materials for several sets of cigarette making trials by Modified Smoking Materials. In addition, twenty-four batches of thermally degraded (15% solids/15 minute hold time) citrus pectin were produced for spray drying and ultrafiltration trials.
- C. **Plans:** In the future, production of batch degraded binder solutions should be greatly reduced, since laboratory measurements have shown the continuously produced binder to meet the required specifications. A sample of the Obipektin degraded apple pectin, that has been tested by FTR, was obtained from Jurgen Krauss. A thorough analysis of the binder will be conducted.

IV. TMCI-ASTA SHEET

- A. **Objective:** To develop a subjectively and physically acceptable reconstituted tobacco sheet using the TMCI process and PM-RCB technology for international application.
- B. **Results:** ASTA products were made in FTR which indicate that no major problem exists obtaining subjective equivalency to US-RCB with this process. These products will not be evaluated for survivability in cigarettes because persistent pinholes in the sheet would overshadow any effect of process changes on sheet integrity. The need for modifications to the design of the casting box and lecithin application system was discussed with TMCI who agreed this would be done before ASTA operations begin in

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Tarragona. It is expected that these modifications and the different dryer design in Tarragona will improve the physical quality of the Spanish ASTA relative to that produced in FTR.

C. Plans:

1. Visit Tarragona in January during Microflake processing to determine the readiness for ASTA operations including analytical laboratory capability.
2. Run ASTA trials in Tarragona in February to determine process conditions for RCB subjective equivalency and optimum sheet quality.

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PROJECT TITLE: Entomological Research
PROJECT NUMBER: 1101
PROJECT LEADER: D. L. Faustini
PERIOD COVERED: October, 1987

I. CIGARETTE BEETLE (CB) PHYSIOLOGICAL STUDIES

- A. Objective: To conduct research investigations designed to produce results that lead to the control of the CB growth and development.
- B. Results: A new method for the treatment of export cut filler that would control the CB has been developed. This method involves the use of ferrous oxide cachets which act as an oxygen absorber. Tests were performed using all life stages of the CB and 100% mortality was obtained after 10 days of observation. This process could be a substitute for the present phosphine treatments for export cut filler.

The warehouse that was treated with CO₂ in early September and then used to store off-shore tobaccos has shown increased numbers of CBs captured in the pheromone traps. This might be due to contaminated tobacco introduced after the CO₂ treatment. The domestic warehouse treated with CO₂ has showed an insignificant number of trapped CBs (1).

A proposal has been written to investigate the use of microbial pathogens that could be used to control the CB. Specifically, Bacillus thuringiensis var. tenebrionis a strain that is pathogenic to beetles (Coleoptera) (2).

- C. Plans: 1) A meeting has been scheduled for November 3 to address the application of ferrous oxide to export cut filler (3). This will include a cost/benefit review; governmental prospective; and a comparison to the present treatment (phosphine). 2) Eight additional tobacco warehouses will be treated with CO₂ in December. 3) A review will be made of the present Bacilli pathogens. 4) A proposal will be written regarding research to be done.

D. References:

1. Minor, M. F. Notebook No. 8539, pp. 14-20.
2. Ryan, L. Memo to D. Ayers, B. Davies, D. Faustini. Working Document to Discuss Bacilli as Potential CB Control Agents. October 2, 1987.
3. Faustini, D. L. Memo to Distribution. Treatment of Export Cut Filler and Other Polyethylene Packages of Tobacco. October 22, 1987.

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II. SERVICES TO OTHERS

- A. Objective: To conduct and provide technical services to areas outside R&D.
- B. Results: Recommendations were made to: QA regarding the optimum (most accurate) phosphine monitoring pumps (1); and, QA regarding atmospheric steaming cycle for improved stems (2).
- C. References:

1. Faustini, D. L. Memo to T. A. Newman. Standard Phosphine Monitoring Equipment. October 2, 1987.
2. Faustini, D. L. Memo to T. A. Newman. Atmospheric Steaming Cycle for Improved Stems. October 12, 1987.

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PROJECT NUMBER: 1620
PROJECT TITLE: Electrophysiological Studies
PROJECT LEADER: F. P. Gullotta
WRITTEN BY: C. S. Hayes
PERIOD COVERED: October, 1987

I. NASAL EVENT-RELATED POTENTIALS (NERPs)

A. Objective: To develop methods by which to objectively and reliably evaluate human responses to cigarettes, smoke constituents and tobacco flavorants.

B. Results:

1. Cognitive NERP Study

Pilot experiments to investigate the basic parameters necessary for recording cognitive NERPs have been initiated with the goal of utilizing these responses to discriminate differences among flavors. Current efforts are focusing on the isolation of the cognitive component of the NERP. Utilizing six second inter-stimulus-intervals (ISIs), experiments are being conducted comparing NERPs when target stimuli are presented sequentially vs when presented randomly in the cognitive paradigm. NERPs to target stimuli in the cognitive paradigm should contain a cognitive component that is not present in the NERPs to target stimuli presented sequentially. To date, it is possible to record responses at six second ISIs using an A/D converter and software furnished by G. Kobal. However, due to problems in the transferring of data into the EPA database, it is currently not possible to retrieve the NERP waveforms for manipulation and evaluation. This problem is currently being investigated by S. Peterson and should be corrected very soon.

Developmental Engineering has begun work on the programming of a reaction time task to be utilized during the cognitive NERP experiments. The task will allow for a signal detection analysis of data gathered from these experiments. Once completed, the program will allow the data from the psychophysical task to be gathered and tagged to the appropriate NERP waveforms under the new ISI conditions.

2. Topographical Mapping Study

More extensive statistical analyses of the data from this study are being conducted in order to compare the findings with results obtained in the Concentration/Response Study. To date, repeated measures analyses of variance have been completed for all NERP waveform measurements (latency and amplitude) comparing left and right nostril NERP data in response to stimulation with vanillin, CO₂, amyl acetate and a mixture of vanillin and CO₂. Post hoc comparisons are currently being conducted for all significant main effects and interactions in order to further examine the sources of the significant differences. All analyses should be completed by early November.

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3. Other Work

Work aimed at determining optimal cleaning methods for materials which might be employed in the yet-to-be-designed olfactometer/smoke delivery system continues. Preliminary investigations conducted with Teflon tubing found the tubing to be contaminated (i.e., smelling of smoke) following a single 50cc, 2.0 second puff of cigarette smoke, suggesting that all subsequent puffs would be contaminated with smoke from the previous puff. Additionally, the results indicated that Teflon is quite permeable to smoke condensate. That is, following cleaning with solvent, tubings appeared to remain clean for only a short period of time before the smoke odor returned, indicating that the smoke was not only contaminating the inner surface of the tubing but penetrating into the Teflon. Most efficient cleaning was accomplished by continuously flushing the tubings with acetone for an interval of time relative to the dimensions of the tubings, followed by continuously sweeping with gas until time for the tubings to be used again. These findings suggest that Teflon is a viable candidate for use in the new smoke delivery system only if the tubings can be changed or efficiently cleaned following one puff of cigarette smoke.¹ Investigations are currently being conducted to repeat these tests using glass and stainless steel.

- C. Plans: Following resolution of the problem in transferring data into the EPA database, plans are to continue pilot investigations aimed at isolating the cognitive component of the NERP. Included in these investigations will be experiments employing two different intensities of the same stimulus, with the high intensity stimulus serving as the target stimulus and the low intensity stimulus, the standard stimulus. The NERP to the high intensity (target) stimulus should contain a cognitive component not found in the NERP to the low intensity (standard) stimulus. Following isolation of the cognitive component of the NERP, experiments will be initiated to systematically manipulate ISIs in order to better understand the mechanisms by which flavor discriminations are made.

D. References:

1. Hayes, C.; Hellams, R. Cleaning of Smoke-Contaminated Teflon Tubing. Memo to F. Gullotta; 1987 October 6.

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PROJECT NUMBER: 1702
PROJECT TITLE: Aerosol Generation and Filtration
PROJECT LEADER: K. Cox
PERIOD COVERED: October, 1987

I. SUBJECT: OPTICAL INSPECTION SYSTEMS

- A. Objective: Develop optical processing techniques for the inspection of cigarette packs.
- B. Results: The acquisition of a video library containing images of both good and defective (fabricated) packs has been completed.

Software routines were written to align (scale, rotate, and translate) images and to form the dot product matrix, A , for any given image set. The elements of this matrix are $A_{ij} = F_i \cdot F_j$, where F_i is a vector of length 512^2 and contains the image intensities. The calculation of the dot product matrix has been carried out for a set of images of both good packs and those with bad tear tape. A synthetic discriminant function (SDF) for discriminating between the good and bad images will be calculated from this matrix using a theory developed earlier. This function will be used to design a matched spatial filter for use in an optical detection system capable of distinguishing between the two pack types.

Two LCD-TV's (made by Epison and Casio) have been modified. The illumination sources, scattering screens, and polarizers were removed and the screens made more homogeneous and optically flat by cementing optical flats cut from holographic film plates onto both sides. An optical processing system was assembled to test the performance of the modified LCD-TV's. When the Epison unit was used to encode images of different cigarette packs on a laser beam, the images produced were found to be of a much higher quality than those produced previously using other LCD-TV's.

One of the most promising optical detection systems currently being considered is based on an optical Hough transformation. The results obtained using this system can be improved significantly if the image is edge enhanced prior to the Hough transformation. We have found that edge enhancement can be very effectively carried out simply by rotating the polarizers used with the LCD-TV's by 45 degrees.

D. Plans:

1. Complete evaluation of the LCD-TV's for use in an optical processing system.
2. Evaluate the Global Holonetics SMART CAMERA. Determine its effectiveness in discriminating between good and defective cigarette packs.

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3. Calculate the synthetic discriminant function (SDF) for the bad tear tape image set. Determine the effectiveness of the SDF for discriminating between the images of good and defective packs.

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PROJECT NUMBER: 1704
PROJECT TITLE: Supercritical Fluid Processes
PROJECT LEADER: J. L. Banyasz
PERIOD COVERED: October, 1987

I. LOW NICOTINE

- A. Objective: Provide on-line nicotine monitoring system for the pilot plant.
- B. Results: Modifications have been completed to permit remote operation of the unit. Problems have been observed resulting from plugging of the sampling line with waxes. Efforts are underway to correct the problem.
- C. Plans: This work is ongoing.

II. LOW NICOTINE

- A. Objective: Pilot plant support.
- B. Results: A series of runs were made in the single absorber mode on the one liter system to determine the effect of citrate loading on absorber performance. The data showed that increasing the citrate level on the stems tends to sharpen the nicotine profile in the absorber. This could be used to decrease the stem requirements somewhat, provided the subjective evaluation of the extracted tobacco is acceptable. The results also suggest that stem solubles play a role in nicotine absorption in addition to the citrate effect.
- C. Plans: This work is ongoing.

III. LOW NICOTINE

- A. Objective: Alternatives to AB.
- B. Results: Extractions were carried out on tobaccos treated with aqueous ammonia at various levels. The results show that increasing the ammonia to twice the level normally added with AB allows the CO₂/tobacco ratio to be reduced by 33% while maintaining comparable extraction efficiency. Subjective evaluation showed that the extracted tobacco fell within the range observed for the AB process.
- C. Plans: This work is ongoing.

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PROJECT NUMBER: 1706
PROJECT TITLE: Thermal and Combustion Processes
PROJECT LEADER: D. B. Losee
PERIOD COVERED: October, 1987

I. OPERATIONS SUPPORT (P. Henderson)

A. Objective: Establish changes that occur in inks as a function of age.

B. Results: Substantial differences in viscosity are evident between a fresh ink and one which is six months old. Their viscosities were determined using a Brookfield viscometer equipped with T-bar spindle. These same inks were tested on an Inkometer (an industry standard instrument) to determine their operating tacks. These two inks also revealed differences in their operating characteristics as determined on the Inkometer.

C. Plans: Further evaluate Inkometer.

II. OPERATIONS SUPPORT (B. Losee)

A. Objective: Establish if particle size analysis provides information on tipping adhesives which can be related to their operating characteristics.

B. Results: Several tipping adhesives were submitted to Brookhaven Instrument Company for particle size analysis. Both a light scattering technique and centrifugation technique were used for this analysis. The latter technique, DCP, involves photosedimentation in a disc centrifuge. This technique shows promise in discriminating among adhesives which have particle sizes that change in time.

C. Plans: Further evaluate the DCP instrument and determine how changes in particle size can affect operating characteristics of tipping adhesives.

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PROJECT NUMBER: 1708
PROJECT TITLE: Physical and Chemical Properties of Tobacco
PROJECT LEADER: M. E. Counts
PERIOD COVERED: October, 1987

I. ALTERNATE HUMECTANTS (J. Crump, E. Mooz)

- A. Objective: To find a plasticizer/humectant system that provides acceptable sheet materials without glycols.
- B. Status: Plans for the Alternate Humectant Program for 1988 have been submitted to management for approval (1). A meeting was held with Park 500 and BL Plant personnel to discuss making PG/G-free sheet (using scrap, isosweet, and potassium propylparaben [K-pp], with no class tobacco, PG, or G). Three modes of operating Park 500 were discussed and the quantity of K-pp required for each mode was estimated (2).

PG/G-free RLTC and RL150B test sheets are being made in C Pilot Plant with two levels of isosweet and K-pp.

- C. Plans: PG/G-free sheets made in C Pilot Plant will be tested chemically, microbiologically, toxicologically, physically, and subjectively prior to production plant runs. Hand-made PG/G-free RCB sheets will be made in C Pilot Plant.
- D. References:
1. Mooz, E., Plans for the Alternate Humectant Program for 1988: Production of a PG/G-free Cigarette, September 25, 1987.
 2. Mooz, E., Alternate Humectant Program Meeting: Use of Potassium Propylparaben in Production Plant Sheets, October 29, 1987.

II. GLYCERIN FREE SHEET/CIGARETTES (J. Crump, E. Mooz)

- A. Objective: To provide acceptable glycerin-free RLTC, RL150B, and RCB sheets and cigarettes for the European market.
- B. Status: C Pilot Plant test sheets of RLTC and RL150B containing various levels of isosweet and PG and no class tobacco or glycerin have been made into 100% cigarettes. Analytical results were obtained.

G-free sheets made at Park 500 and the BL Plant have been made into 100% cigarettes. Analytical testing was completed. G-free sheets were also used in blended cigarettes using all G-free tobacco components and casings (in collaboration with J. Hickie).

- C. Plans: G-free test sheet 100% cigarettes will be submitted for subjective evaluation. G-free blended cigarettes will be submitted for subjective evaluation when analytical testing is completed.

III. LOW DENSITY RODS (S. Ganeriwala, M. E. Counts)

- A. **Objective:** To compare compression properties of individual low density and standard density rods and to measure compression responses of control and test filler used in these rods as a function of moisture and temperature.
- B. **Status:** Constant compression rate testing at standard conditions of low density rods from a binder concentration study is nearly complete. Average initial force-displacement slopes at low loads generally decrease with decreased binder level for 580mg and 725mg target rods. There were inconsistencies for the 650mg target rods. Forces at 1, 1.5, 2, and 3.5 mm displacement followed these trends. Additional testing will be done to verify these results.

A second set of test rods is also being studied. This set includes standard density rods made with control, water sprayed, and unactivated binder-applied filler. Low density rods with different weight reductions are also included.

- C. **Plans:** Continue constant rate compression testing of the above described materials.

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PROJECT NUMBER: 1720
PROJECT TITLE: Analytical Microscopy
WRITTEN BY: V. Baliga
PERIOD COVERED: October, 1987

I. STUDIES IN MICROSCOPY

- A. Objective: Examine the ultrastructure of selected cigarette papers in support of the low sidestream project.

B. Results:

Plans were finalized to study the ultrastructural changes (1) and the macro and micro changes of the paper cross section (2) that occur in low sidestream (LSS) cigarette papers as a function of temperature (1). The first study is a collaborative effort between Ken Maloney and Karen Sanders. The second study will be completed by Vicki Baliga.

An experimental LSS paper from Kimberly Clark made with small particle size chalk and monoammonium phosphate was examined using STEM. It contained Ca particles of very small size. The particles did not encase the fibers (3).

A 100% flax paper with no additives was examined as a control using STEM. The separation of flax fibers into smaller, individual fibrils could be seen (4).

- C. Plans: Seven LSS papers will be analyzed by microscopy after heating to 3 temperatures plus ambient. Microscopic examination will include fiber and crystallite structure (1, 2).

II. RESPONSE TO ANALYTICAL REQUESTS

- A. Objective: Provide analytical support to R&D.

B. Results:

In support of project QUICK two carbon rods were analyzed by EDS. One was received from T. Sanders and was described as a 'handwarmer' carbon rod having a "silver" paint on its outer surface. The inner and outer surfaces were analyzed for heavy metals (5). The only elements found on the inner surface were Si, K, and Ca. The outer surface elements were Al, K, and Ca. The "silver" paint was aluminum based. The second carbon rod was described as an extruded carbon rod bound with polyvinyl alcohol and then heated to drive off the PVA. EDS analysis was performed on both the inner and outer surfaces of this sample (6). Outer surface elements found were Mn, Si, S, P, K, Ca, Mg, and Cl. Inner surface elements were Al, Si, S, K, Ca, Ti, Mn, Fe, and P. The sample exhibited a great deal of elemental variation within and between areas analyzed.

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A red pellet of unknown composition and structure, located in the center of a smoked cigarette filter, was submitted by B. Fenner for analysis. EDS was completed on the inner and outer surfaces of the pellet. No elements above Na were detected. Microscopic examine of the pellet showed that it contained cavities filled with red material throughout the polymer matrix. The pellet also had an additional layer coating the outer surface (7).

A commercial Smoke-Check CO monitor was submitted by Gen Baker for EDS and structural analysis. The 2 sealed standards strips contained Ti, Ca, Si, and some Al. The 0.1 standard also had a small amount of Fe. These materials consisted of a fine granular matrix. The CO monitor strip contained Al as the support material with S, Ca, Cl, and Pd. The Al support matrix consisted of coarse, much larger particles than the standards strips (8).

A metal coupon with black particles was submitted for analysis by Duane Watson. The coupon had been placed above the stems in the ART pressure vessel. The black particles were separated from the metal coupon by sonicating the coupon in methylene chloride. The particles were paramagnetic and the majority of particles contained Fe, Cr, and Ni and were flat plate-like structures. Some were round and contained only Fe (9).

A new system has been established for incoming sample requests to the Analytical Microscopy group (10). This system will provide microscopists with better information about samples to be analyzed. It also provides a means of establishing an in-house data base (central file) as well as a mechanism of incorporating appropriate samples into a directory of microscopy on tobacco and tobacco related materials.

C. References:

1. Baliga, V., "Proposal - Paper Degradation Studies as a Function of Heat: I. Ultrastructural Characterization," memo to Richard Cox, October 27, 1987.
2. Baliga, V., "Proposal - Paper Degradation Studies as a Function of Heat: II. Ultrastructure of Fiber Cross Section and Void Volume," memo to Richard Cox, October 27, 1987.
3. Sanders, K., P.M. Notebook #8374, p. 155.
4. Baliga, V., P.M. Notebook #8412, p. 102.
5. Sanders, K., P.M. Notebook #8374, p. 147.
6. Sanders, K., P.M. Notebook #8374, pp. 148-149.
7. Baliga, V., Sanders, K., "Analysis of Red Filter Pellet," memo to R. Cox, October 15, 1987.
8. Baliga, V., P.M. Notebook #8412, p. 98.
9. Baliga, V., "Debris From ART Pressure Vessels," memo to Duane Watson, October 23, 1987.
10. Sanders, K., "Requests for Analytical Microscopy," memo to Distribution, October 9, 1987.

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PROJECT NUMBER: 1730
PROJECT TITLE: Plant Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
PERIOD COVERED: October, 1987

I. TOBACCO-IDENTICAL PRESERVATIVES

A. Objective: To develop procedures and to establish microbiological screens for the evaluation of new, tobacco-identical (or "nature-identical") preservatives as replacements for and/or adjuncts to propylparaben.

B. Status:

1. Microbiological Screens: [M. A. Shulleeta, S. W. Tenhet]

- a. Shake-Flask Assay: A synthetic sucrose monoester (obtained from Dr. G. Chan) was evaluated for antimicrobial activity in our standard, Phase I shake-flask preservative screen using *B. coagulans* as the test organism and 150 µg/ml propylparaben in propylene glycol (PP-PG) as the control.
- b. Agar Diffusion Assay: A standard operating procedure is being developed for an alternative preservative screening assay in which a freshly established lawn of a test microorganism is challenged with a prospective preservative candidate that has been applied at various concentrations to small (10 mm) filter paper discs. If the test microorganism is sensitive to the compound under evaluation, a clear (cell free) "zone of inhibition" will form around the filter paper disc containing that material (the diameter of the zone generally being related to the relative antimicrobial activity and solubility of that compound).

2. General Microbiology: [J. L. Lyle, L. Weissbecker]

- a. Sporulation: Work continues on the development of a standard operating procedure for the storage of bacteria as spores and/or as freeze-dried vegetative cells.
- b. Microbial Identification: An evaluation of the VITEK microbial identification system is being conducted in collaboration with members of Project 1902.
- c. Isolation of Bacteria from Park 500 SEL: Five pure cultures of Gram Positive bacteria that were isolated from Park 500 SEL have been sent to the American Type Culture Collection (ATCC) for identification.

3. Extraction of Oriental Tobacco: [D. M. Teng]

An oriental tobacco (LSB) rich in β -methylvaleric acid was used for the isolation and fractionation of surface and cytoplasmic

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lipids for evaluation as potential antimicrobial agents in the paraben replacement program.

4. Preservatives List: [I. L. Uydess]

A list of alternative, preservative candidates has been compiled based upon an evaluation of naturally-occurring compounds (including essential oils and essential oil components) that are known to be approved for use in food-grade and/or tobacco products as a result of their inclusion in one (if not more) of several published approved additives lists (such as the USDA Title-21, UK-Hunter and Fema lists, etc.). A memo describing the rationale of selection and plans for the testing of these compounds has been completed and will be distributed by the end of October.

C. Results:

1. Microbiological Screens:

- a. Shake Flask Assay: The synthetic sucrose monoester was tested in duplicate experiments in the Phase I (Nutrient Broth, pH 5.7, 37°C) screen at 50, 250, 500, 1000, 5000, and 10,000 µg/ml. No antimicrobial activity was observed at any of the concentrations evaluated compared to the half-maximal inhibition of growth that was observed in the standard 150 µg/ml PP-PG control.
- b. Agar-Diffusion Assay: Several important experimental parameters were evaluated with regard to establishing an S.O.P. for this assay. Three agar formulations (Nutrient Agar, Trypticase Soy Agar, and Oxoid 'D.T.S.' Agar) were compared using various serial dilutions of the overnight (stationary) cultures of four Gram Positive bacteria (B. coagulans, B. licheniformis, B. subtilis, and B. pumilis). Three known antimicrobial compounds (Penicillin, Streptomycin and Chloramphenicol) were employed as the "test" preservatives. Nutrient Agar was generally found to provide the most uniform growth (overall) for the various bacteria examined and yielded the most defined zone of inhibition around the antibiotic discs that were used. A dilution of 1:100 of the overnight cultures of each organism was also found to produce the optimum lawn of bacteria for testing (0.3 ml of this dilution being used to inoculate each 100 mm diameter plate). Furthermore, the preliminary protocol that was generated was successfully tested against B. coagulans using several concentrations of PP-PG applied to sterile filter paper discs.

2. General Microbiology:

- a. Sporulation: Four Gram Positive bacilli known to be associated with tobacco products have been sporulated and stored for future use (B. coagulans, B. licheniformis, B. subtilis, and B. brevis).

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- b. Microbial Identification: Growth curves have been generated at 37°C and 60°C for six ATCC type strain cultures of bacilli in order to more accurately establish their optimum growth temperature (e.g., as either mesophiles or thermophiles) so that they can be used in a further evaluation of the Vitek microbial identification system. Of the organisms tested thus far, only B. coagulans appears to be a true thermophile based upon the higher growth rate and final (stationary phase) density obtained at 60° vs 37°C.

3. Extraction of Oriental Tobacco:

The surface lipid was removed from the surface of cured samples of oriental (LSB) tobacco using methylene chloride. The resultant extract was subsequently partitioned in hexane and aqueous methanol, and the methanol phase extracted with chloroform. The chloroform extract was then fractionated on a Sephadex LH-20 column. Eight fractions were recovered and stored frozen until they can be tested in future Phase I antimicrobial screens. After the removal of the surface lipids, the leaves were blended in a Waring blender with hexane followed by methanol to extract the cytoplasmic lipids. The combined extract was further extracted with ether and partitioned in hexane and aqueous methanol. Three crude fractions of these cytoplasmic lipids were collected and stored for future testing.

D. Conclusions: None to be reported at this time.

E. Plans:

1. Additional prospective preservatives will be tested in the Phase I microbiological screen during the remainder of the 4th Quarter, 1987. Target compounds will include sucrose esters and crude extracts isolated from oriental tobacco as well as "nature-identical" compounds that have been approved for use in tobacco and/or food-grade products.
2. A standard operating procedure for the agar diffusion assay will be completed by the middle of November, 1987. This procedure will be employed in the evaluation of prospective preservative compounds as an adjunct to the Phase I (Nutrient Broth) screen that is currently in use.
3. Work will be initiated on the development of a Phase II (SEL) screen for use in future antimicrobial/preservatives tests.
4. The collaborative evaluation of the VITEK microbial identification system will continue until the end of the 4th quarter in support of the objectives of Project 1902.

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PROJECT NUMBER: 1754
PROJECT TITLE: Spectroscopic Studies of Tobacco and Smoke Components
PROJECT LEADER: J. B. Wooten
PERIOD COVERED: October, 1987

I. SOLID STATE NMR

A. Objective: Characterize tobacco and related products by ^{13}C CP/MAS NMR.

B. Results: Two Japanese RL products were examined by solid state NMR for the presence of higher than average levels of cellulose. The C-4 signal of cellulose is free from interfering signals in intact and composite plant materials. The intensity of this signal can be used to make a crude estimate of the relative amount of cellulose present. Both Japanese RL products exhibit enhanced signals for C-4 at 89 ppm relative to Philip Morris RL. One sample contains at least twice the PM level of cellulose. This same sample exhibits an unusual carboxyl region signal at 179 ppm which is not typical of either intact plant or composite plant materials. Carboxymethyl cellulose, which is commonly used as a binder, exhibits a strong carboxyl signal at 178 ppm, and is being investigated as the possible source of this unusual signal.

A. Objective: Determine the local environment and mobility of nicotine in tobacco in situ.

B. Results: A detailed, technical memo describing the use of isotopic labeling and solid state NMR for the detection of nicotine in tobacco was recently issued.

C. Reference:

Memo from J. Wooten to R. Izac, "In Situ Detection of Nicotine in Tobacco by ^{13}C CP/MAS NMR", October 21, 1987.

II. SOLUTION NMR

A. Objective: Determine the degree of esterification of degraded pectins.

B. Results: We are currently using ^{13}C solution NMR to determine the per cent of methyl esterification in degraded pectin samples submitted by Sherry Baldwin. The degree of esterification is obtained by integrating the carboxyl region signals which exhibits well resolved signals for the esterified and free carboxyl groups. The NMR results compare favorably with those obtained by the titration method. The availability of different pectin samples that have been degraded to varying extent affords the opportunity to look for differences in their ^{13}C spectra that might result from the variation in the polymer chain length. At least five distinct spectral patterns have been identified for different pectin samples in the region between 95 and 110 ppm that contains the anomeric

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carbon signals. As many as five, or as few as two groups of signals appear in the spectra of different samples. These differences may be useful for characterizing the extent of degradation in pectin being prepared for use as a foamed binder.

- C. Plans: The signals in the anomeric region of the degraded pectins will be identified and correlated with pectin structure.

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PROJECT NUMBER: 1757
PROJECT TITLE: Analytical Flavor Specifications
PROJECT LEADER: C. S. Kroustalis
PERIOD COVERED: October, 1987

ANALYTICAL FLAOUR SPECIFICATIONS

- A. Objective: To develop analytical and subjective specifications for incoming flavors and materials used at the Flavor Center and other QA facilities.

B. Results:

Analytical and sensory evaluations were completed for thirty-five of the 87 German market materials (~ 40%). Approximately 20-25 additional materials (24-28%) should be completed within two weeks. The rest of the materials on hand (12-14 samples and retains) and an additional 17 materials on order should be completed by mid to late December. Component identifications have a small lag time because of the sheer volume of materials received within this past month, but we do not anticipate any appreciable delays.

CAD plans to upgrade Nelson hardware which will also include a PC network. These improvements will speed up analytical data processing and handling. We appreciate their efforts.

- C. Plans: Complete the analytical/sensory evaluations of approximately thirty-five materials on hand and begin the evaluation of the seventeen materials on order. The status for the total project by the end of 1987 is estimated to be about 38% complete. We additionally plan to transfer evaluations of 6 materials to the Flavor Center within a week.

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PROJECT NUMBER: 1758
PROJECT TITLE: Tobacco Cell Wall Research
PROJECT LEADER: G. H. Bokelman
PERIOD COVERED: October, 1987

I. BLEND COMPOSITION ANALYSIS (G. Bokelman, J. Stimler and
General Analytical)

- A. Objectives: Predict the blend compositions of Camel (non-filter, 70 mm), Camel Filter (85 mm), Capri Menthol, Capri Regular (separate samples obtained in February and April), Caprice and Slim Line cigarettes.
- B. Caveats: Our standard method of estimating blend compositions based upon chemical predictors should give accurate results when the actual tobacco components to be used are incorporated into the database, a condition which for practical purposes only can be met for products manufactured by Philip Morris. Inaccurate results may be obtained if this method is applied to unknown blends or blends whose components differ markedly in chemistry from those used in the database. The database for this study consisted of typical commercial tobacco components used by Philip Morris for domestic cigarette production in the first quarter of 1987.

C. Results:

The predicted blend compositions for the various cigarette brands are reported below. Values are expressed as percentage of total, rounded to the nearest percent.

<u>Component</u>	<u>Camel</u>	<u>Camel</u> <u>Filter</u>	<u>Capri</u> <u>Menthol</u>	<u>Capri</u> <u>Reg.</u> <u>(2/87)</u>	<u>Capri</u> <u>Reg.</u> <u>(4/87)</u>	<u>Caprice</u>	<u>Slim</u> <u>Line</u>
Bright Lamina	48	41	57	47	42	43	64
Burley Lamina	25	17	16	28	26	25	16
Oriental Leaf	8	18	17	20	22	20	9
Stem	19	24	10	6	9	12	11

Previously, based solely on β -methylvaleric acid determinations, it was estimated that Camel contains 9.3% Oriental leaf and Camel Filter contains 19.0% Oriental leaf.

- D. Plans: In collaboration with Bill Ryan and Betty Handy, chlorogenic acid and rutin will be evaluated as potential predictors for bright lamina content.

E. Reference:

Bokelman, G. H. and J. O. Stimler, memo to Dr. J. L. Charles, "Estimations of Blend Composition of Capri, Caprice and Slim Line," October 8, 1987.

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II. MOLECULAR WEIGHT DISTRIBUTION OF DEGRADED PECTIN SAMPLES (S. Baldwin)

- A. Objective: Evaluate the molecular weight distributions for a number of degraded pectin samples by gel permeation chromatography in order to compare samples prepared by different processes and to examine the consistency of individual processes.

B. Results:

Medium pressure gel permeation chromatography was performed using 0.1 M phosphate buffer at a pH of 6. Dextran standards were used to calibrate the columns for weight average molecular weight. An excellent linear calibration was obtained; however, it should be noted that the use of dextran standards for the estimation of pectin molecular weight will be biased to yield high values because pectin and dextran do not have the same shape in solution.

In-house thermally degraded pectin samples from the continuous process were compared to batch degraded samples. In general, the continuously degraded materials appear to have small amounts of higher molecular weight components than the batch degraded samples. None of the continuously degraded samples was a perfect match for SD-14, the currently preferred (in-house) binder. SD-14 was prepared in the batch process by holding a 15% solution of pectin at 145° C for 15 minutes.

Three experimental samples obtained from Hercules were evaluated. These samples were all very different from one another, indicating that neither the process conditions nor the product are yet well defined. Two of the samples had lower molecular weights than our in-house degraded samples.

A comparison also was made between Grindsted samples TSP-092 and TSP-095, which represent different batches of thermally degraded lime pectin. The profiles for these two samples were virtually identical, indicating that Grindsted has very good control of their degradation process.

Finally, the reproducibility of the Philip Morris batch process was examined. SD-33 was made under the same process conditions as SD-14, that is a 15% pectin solution was held at 145°C for 15 minutes. Comparison of the respective chromatograms showed that the major component elutes in fraction 22 for both samples. The overall elution profiles also are very similar, indicating that the process conditions have been well controlled.

- C. Plans: A limited number of other degraded pectin samples will be analyzed, as time permits.

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PROJECT NUMBER: 1902
PROJECT TITLE: Tobacco Microbiology
PROJECT LEADER: D. J. Ayers
WRITTEN BY: D. K. Chadick
PERIOD COVERED: October, 1987

I. PROJECT ART BLEND SAMPLES

- A. **Objective:** To determine the potential for bacterial and fungal growth in the ART total blend tobacco samples at various OV's during storage at 108° F and to monitor any chemical changes that may occur.
- B. **Results:** MC Primary facility samples (OV 20 - 21%) were collected and tray dried to various OV's. Samples were placed in containers and microbially and chemically analyzed after 0,4,8,12,16,24 and sometimes 48 and 72 hours of storage at 108°F. As previously reported (1), the results from all the samples held at OV levels less than 16% did not exhibit an increase in microbial counts (>10 fold increase as compared to starting counts). The chemical changes, primarily in malic acid, did not appear to correlate well with the microbial counts (2). A memo was issued with the recommendation to hold the ART blend at a target %OV of 13.5% ± 1% (3).
- C. **Plans:** A memo with the final results from this study is in preparation.
- D. **References:**
1. Mallory, O. Project 1902 September Monthly Summary. PM Monthly Summary, Acc. No. 87-100.
 2. Turner, D. PM Notebook No. 8285.
 3. Ayers, D. Recommended %OV for Total Blend Strip (Project ART). Memo to B. Sorrels; 1987 October 8.

II. MICROBIAL ANALYSIS OF PECTINS FROM THE REDUCED DENSITY ROD PROGRAM

- A. **Objective:** To microbially analyze the 2% Bulmer and 8% Genu pectin solutions and Marlboro and lamina blends coated with and without either pectin. This analysis will cover storage at 25°C and 4°C for up to four months.
- B. **Results:** The yeast and mold counts from all blend samples held at 25° and 4°C remained in the acceptable range after 49 and 63 days of storage. Pectin samples (results previously reported (1)) were not collected after day 35. The bacterial results from the blends continued to demonstrate variability with regard to increases and decreases in bacterial numbers after 49 and 63 days of storage (2).
- C. **Plans:** This is an ongoing study.

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D. References:

1. Mallory, O. Project 1902 September Monthly Summary. PM Monthly Summary. Acc. No. 87-100.
2. Mallory, O. PM Notebook No. 8505

III. BACTERIAL IDENTIFICATION

A. Objective: To determine the best method for identifying micro-organisms isolated from various tobacco products.

B. Results: Growth curves at 37.5° and 60°C in T-Soy Broth using ATCC cultures Bacillus circulans, B. subtilus, B. licheniformis, B. megaterium, B. pumilus, B. polymyxa, and B. coagulans have been performed to demonstrate the optimum growth temperature of these bacteria (1,2).

C. Plans: These data will be used to further analyze the cultures with the Vitek (leased for 3 months), the API Rapid CH strips, and other biochemical tests.

D. References:

1. Chadick, D. PM Notebook No. 8181.
2. Lyle, J. PM Notebook No. 8308.

IV. ALTERNATE HUMECTANT PROGRAM

A. Objective: To determine the effects of storage time and temperature on RL and RCB production plant material produced as part of G-free portion of the Alternate Humectant Program.

B. Results: To date, following 4 weeks of storage, the the microbial numbers from a majority of the the sheets (tests vs. controls) showed little change when compared to the time zero numbers (1).

C. Plans: This is an ongoing study. A memo will be issued with the final 12 week storage results.

D. References:

1. Crockett, E. PM Notebook No. 8563.

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PROJECT NUMBER: 1904
PROJECT TITLE: Tobacco Physiology and Biochemistry
PROJECT LEADER: H. Y. Nakatani
PERIOD COVERED: October, 1987

I. LOW NICOTINE STUDY

- A. Objective: To investigate the biochemistry of the nicotine biosynthetic pathway at putrescine N-methyltransferase (PMT) and N-methylputrescine oxidase (MPO) and specifically to isolate PMT from tobacco root extracts.
- B. Status: The roots from the hydroponically-grown, Burley 21 (Bu21) tobacco plants of group six have been harvested (1,4). Three extracts from group six have been processed to obtain the 40-65% ammonium sulfate fraction (1,4). The remaining extracts from group five were fractionated by the 3 column purification series cited in last month's summary. The PMT-active fractions from the phenyl-Sepharose columns were pooled and concentrated with 65 % (saturation) ammonium sulfate. A fourth (DEAE-Sepharose) chromatographic step was also examined, with the pooled PMT-active samples from the above 3 column scheme (2,3). One of the most PMT-active fractions from this last DEAE-Sepharose column (concentrated against PEG) was examined by SDS-PAGE and 5-7 bands were visualized by Coomassie Blue staining (about 12 bands were observed by silver staining) (5). A major band was observed in the 45-60 kDa molecular weight region (the reported molecular weight for PMT is 60,000). Samples surrounding the most PMT-active fraction from the DEAE-Sepharose column were also concentrated and examined by SDS-PAGE (5). Only 1-3 bands, dependent upon the sample, were evident by Coomassie Blue staining for those fractions from this latter column. Upon silver staining the total number of bands that were detected was increased; however, only 2-3 major bands were observed.

A larger gel filtration column (Sephadex G-100, 1 X 40 cm) has been prepared and is being calibrated to determine the molecular weight characteristics of PMT (6). The separation volume for Blue dextran (mw > 2 million, for void volume determination) and cytochrome c (about 12,500 mw) was increased from about 7.5 mL for the previous (1 X 20 cm) column to about 14.5 mL for this column. This increased separation volume should be of value for the examination of the molecular weight and gel filtration characteristics of PMT. PMT-active fractions which were obtained from the FPLC Mono Q (anion exchange) column were re-applied to the Mono Q column after buffer exchange (4). Two PMT-active peaks were obtained at 30 mM and 200 mM NaCl. The PMT-active fractions from the 200 mM NaCl peak fractions from two runs were pooled, buffer exchanged and re-applied to the Mono Q column. Several peaks were obtained through the application of a modified gradient elution. Weak PMT-activity was observed at an elution profile other than 200 mM NaCl. A literature search by Pharmacia upon our request indicated that heterogeneity has been evident for the Mono Q column, i.e., more iso-electric forms observed from Mono Q than by isoelectric focusing. Our results and the literature information indicate

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considerable development is required before the Mono Q column can be used routinely, and it has been discontinued at this point in our investigations.

- C. Plans: The harvest of the 6th group of hydroponically-grown tobacco roots will be initiated. The three column purification scheme will be used to produce a continuous amount of PMT-active protein for further work to purify PMT. The DEAE-Sepharose column will also be implemented in the purification scheme. The new gel filtration column will be used to examine the molecular weight and gel filtration characteristics of PMT. Work will also continue to produce enough sample for antibody production and screening. Binding studies with protein-A agarose beads, PMT containing extracts, and PNMT antibody will continue.

D. References:

1. Dunn, R.L. PM Notebook No. 7899.
2. Malik, V. S. PM Notebook No. 8402.
3. Nakatani, H. Y. PM Notebook No. 8384.
4. Sherwood, K. R. PM Notebook No. 8216.
5. Sykes, A. PM Notebook No. 8526.
6. Yu, T. PM Notebook No. 8381.

II. OTHER PROJECT ACTIVITIES:

- A. TCRC presentations were given by Ved Malik, Elizabeth Mooz, Karen Rapp Sherwood and Herb Nakatani at the 41st TCRC held in Greensboro, NC (Oct. 3-7, 1987). Reviews of assigned papers were written and presented to Carol Lunsford.
- B. Bruce Davies and Herb Nakatani visited with officials of Hazelton Biotechnologies Company (Vienna, Va) on October 9, 1987, to discuss immunization schedules and development of monoclonal antibodies for our studies.

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PROJECT NUMBER: 2500
PROJECT TITLE: Fundamental Chemistry
PROJECT LEADER: J. I. Seeman
PERIOD COVERED: October, 1987

I. FLAVOR/ODOR CHEMISTRY (Paine, Secor, Seeman)

- A. Objective: To develop new technologies for smoke deliveries of desired flavorants; to prepare new substances for flavor/odor evaluation; to develop methodologies for the analysis of subjective data; to derive relationships between physicochemical parameters and subjectives.
- B. Results and Plans: Excellent progress is being made toward the synthesis of large quantities of all seven configurational stereoisomers of menthol. Pure (99.3+%) samples of d-Neoisomenthol and d-Neomenthol, were prepared and submitted for evaluation by the Odor Profiling Panel and, along with recrystallized d-isomenthol, to Flavor Development. A large scale preparation of pure d-menthol from di-d-menthyl 2,6-naphthalene dicarboxylate was effected, preparatory to oxidation to d-menthone. To improve the equilibrium content of d-isomenthone, l-menthone was epimerized (over K_2CO_3) at its boiling point, as a model reaction. The d-isomenthone content (reportedly 29% at room temperature), was increased to 37%.

As the first step in enhancing our patent position on the flavor release of substituted heterocycles in addition to pyridines and pyrazines, two trimethylisoxazole release agents were prepared. Initial pyrolysis studies are in progress. In addition, the two anethole release agents whose smoke chemistry (SS/MS) was recently characterized by S. Tafur and B. Levins are being examined by competitive pyrolysis.

II. APPLICATIONS OF THEORETICAL CHEMISTRY (Kao, Seeman)

- A. Objective: Develop the necessary algorithms and software to study physical properties of flavor and tobacco related molecules; utilize these techniques to model tobacco smoke and pyrolysis chemistry.
- B. Results and Plans: Efforts have been in extending the MOMM approach to nitrogen-containing compounds. Successful progress has been made in expanding the MOMM method to C-nitroso compounds and nitrites. Ab initio calculations of models for C-nitroso compounds and nitrites are near completion.

We have initiated theoretical mechanistic studies of the glycerol-acrolein reaction. Semi-empirical calculations of the ground-state structures and energies for model compounds were completed. Efforts are now concentrated in locating relevant transition-state geometries. Catalytic effects such as $Mg(OH)_2$ on ground and transition states will be investigated.

MNDO molecular orbital theory has been systematically employed to investigate the pyrolysis mechanisms of β -hydroxypropanal, β -hydroxypropionic acid, and β -hydroxypropionate. These serve as models for various flavor release systems.

A pseudo cyclic 6-membered transition state is confirmed in all three cases. MNDO results provide the adequate data for further refinement and higher level of studies. A complete report has been issued.

MOMM calculations were performed on a variety of alkyl substituted benzenes which are being studied with E. R. Bernstein.

III. CHEMICAL PHYSICS STUDIES OF TOBACCO CONSTITUENTS (Secor, Seeman)

- A. Objective: To obtain structural information about important tobacco constituents/flavorants; to develop information on cluster and aerosol formation and chemistry.
- B. Results and Plans: The supersonic molecular jet spectroscopy (SMJS) study of alkylbenzenes is essentially complete. Anethole and related styrenes are being investigated, and preliminary experiments have been started to determine if chemical reactions in clusters can be observed for the first time. The reaction of anethole with water has been investigated and preliminary results are very interesting.

A number of substrates for examination by SMJS have been prepared, namely 4-dimethylaminobenzotrifluoride and 4-di-trideuteromethylaminobenzonitrile.

IV. MISCELLANEOUS (Leister, Secor)

- A. Objective: To convert important programs of the Chemical Research Division from the DEC system to the UNIX system.
- B. Results: A log of current users of the DEC system for this Division was obtained from CAD and work has begun in converting the remaining programs and files to the UNIX system.

A total of twelve synthetic samples, mostly tobacco alkaloids, were given to various PM researchers in support of their work.

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PROJECT NUMBER: 2501
PROJECT TITLE: Smoke Chemistry
PROJECT LEADER: R. A. Comes
PERIOD COVERED: October, 1987

I. SIDESTREAM SMOKE

- A. **Objective:** Conduct analyses on mainstream and sidestream smoke to determine qualitative and quantitative differences between control and reduced visibility cigarettes. Develop methods to analyze for selected components. Demonstrate effective methods for reduction or removal of these components.
- B. **Results:** Sidestream visibility, particle size, particulate generation rate, mass burn rate and relative particulate fraction data have been obtained on the cigarette models described in the September monthly report. These cigarette models were manufactured to address the effects of circumference and $Mg(OH)_2$ wrappers on mainstream and sidestream smoke chemistries and on sidestream visibility. GC/MS analyses of sidestream and mainstream smoke from these models is ongoing. RS/1 is being utilized to analyze component variability and run to run reproducibility. Individual sidestream component analyses are being carried out by the Analytical Research Division.

A new scheme has been investigated for analyzing sidestream smoke. The procedure consists of collecting a portion of sidestream smoke from the total smoke generated during static burn of four cigarettes onto a column of silica gel followed by elution of the silica gel with hexane and then with ethyl acetate. The eluted fractions are then gas chromatographed with detection by NPD, FID and MSD. This procedure is quick, simple and reproducible. Since multiple separation schemes are used, portions of sidestream smoke not previously observed using more direct sampling and analysis schemes are seen.

- C. **Conclusions:** Visibility measurements indicate the expected decrease in visibility at a given circumference by replacement of conventional wrappers with $Mg(OH)_2$ wrappers. A small, but significant, visibility reduction is evidenced with reduced circumference cigarettes at constant blend and the same wrapper. As expected, additional reduction occurs in the 17mm circumference cigarettes with low visibility wrappers.

Sidestream smoke from cigarettes with and without 35% $Mg(OH)_2$ wrappers have been investigated by the new separation scheme. Elution with ethyl acetate gave fractions which contained numerous nitrogenous compounds, including nitriles, pyrazines, pyridines, pyrroles, etc., moderately polar unsaturated materials, many ketones but few acids or aldehydes. This fraction smelled much like cigarette smoke. A total of 125 individual components have been identified in these fractions. Some reproducible but relatively small differences can be seen between the two cigarette models. A re-run of the samples after standing for a week yielded

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additional small differences. These may correlate with a somewhat noticeable odor difference and with the obvious formation of a colored layer on the walls of the glass vials.

- D. Plans: Continue GC/MS studies on the total mainstream and sidestream smoke from the cigarette models with different circumferences and with and without $Mg(OH)_2$ wrappers.

Investigate the hexane fraction generated by the new separation technique and initiate efforts to analyze that portion of sidestream smoke not trapped on the silica gel column. Consider additional solvents and other materials for separation and trapping of sidestream smoke.

In conjunction with the Analytical Research Division, continue the studies to determine levels of certain components of sidestream smoke.

PROJECT NUMBER: 2507
PROJECT TITLE: Radiochemical Investigations
PROJECT LEADER: S.B. Hassam
PERIOD COVERED: October, 1987

I. Sidestream Gas Phase Composition

- A. Objective: Develop methods for sidestream gas phase collection, sampling and analysis of cigarettes spiked with ^{14}C -glycerol.
- B. Results: Plans have been made to modify smoking machines for gas phase collection from radiolabeled cigarettes. Initial investigations will focus on using (1) single port impaction machine (2) single port syringe type machine and (3) total recovery single port machine. At the recent 41st TCRC meeting W.E. Crouse et al. of Lorillard presented a paper (#25) on collection devices for use in determination of formaldehyde in SS smoke. A copy of the manuscript has been requested as some of the collection designs will be useful in our work with radiolabeled cigarettes.

Purchase of two types of radioactivity gas detectors, flow-through and stop-flow, is under consideration.

Radioanalysis by TLC and paper chromatography of ^{14}C -glycerol obtained from NEN gave confusing results. A batch of ^{14}C -glycerol was obtained from Amersham Corp. Both radiolabeled samples were observed to chromatograph in the same way i.e. in some chromatography systems either partitioning or adsorption led to double peaking and/or broadening. The overall conclusion is that the radiochemical purity of both materials is in excess of 97%.

- C. Plans: Continue the above investigations.

II. SMOKE CHEMISTRY STUDIES

- A. Objective: Determine distribution into smoke from cigarettes labeled with ^{14}C -glycerol.
- B. Results: 40 g of sieved manicured 2R1 filler was sprayed with 283.02 μCi of ^{14}C -Glycerol in 5 mL water:ethanol (1:1).
- C. Plans: Prepare cigarettes for smoking studies.
- D. References: N.B. 8361.

III. OTHER ACTIVITIES

- A. Biosynthesis of ^{13}C -labeled sucrose esters has been initiated (memo from R. R. Izac and R. H. Newman, Protocol for harvesting tobacco fed ^{13}C - β -methylvaleric acid, Oct. 2, 1987).

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B. The following papers were presented at the 41st TCRC, October 4-7, 1987:

1. Use of a Schlieren Optical System to Observe the Thermal Column Rising from a Cigarette Coal. D. D. McRae and R. W. Jenkins, Jr.
2. The Detection of Carbon-14 in Individual Aerosol Particles. R. W. Jenkins, Jr. and D. D. McRae.
3. Cigarette Smoke Transfer Studies. Transfer of Added [18-¹⁴C]Octatriacontane. R. T. Bass, L. E. Brown, S. B. Hassam, G. C. Newell, Jr. and R. H. Newman.

C. A trip report of the 1987 meeting of the American Association for Aerosol Research was issued (memo from S. B. Hassam, October 15, 1987).

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PROJECT NUMBER: 2520
PROJECT TITLE: Flavor Research
PROJECT LEADER: E. W. Southwick
PERIOD COVERED: October, 1987

I. MENTHOL RELEASE CHEMISTRY

- A. Objective: To develop menthol-release flavorants.
- B. Results: Pyrolysis and LTF smoking experiments have shown that, among the components of the present menthyl glucose carbonate mixture, two compounds are significantly more efficient for menthol release. These are 1,6-di-O-menthyloxycarbonyl- β -D-glucose and 1-O-menthyloxycarbonyl- β -D-glucose. A series of experiments has been completed which demonstrate that these two components can be increased to comprise 60% of the mixture instead of the present 27%. A modification of the present synthesis could provide a mixture which delivers menthol more efficiently.

II. PROJECT EXTRA

- A. Objective: To develop proprietary flavor additives for enhanced flavor perception in low delivery cigarettes.
- B. Results: The increased commercial availability of alpha- and beta-cyclodextrins now warrants a re-investigation of their use in flavor release systems. Anethole has been successfully complexed with the cyclodextrins to give an odorless solid; when treated with water or glycerine, the characteristic odor of anethole is clearly perceived. Attempts to form similar solid complexes with flavor mixtures or tobacco volatiles were not successful; in these cases, spray drying or freeze drying the aqueous solutions may be necessary.

The investigation of structural modifications of hexahydro-2,3H-benzofuranones has been extended to the preparation of a 6-n-butyl derivative. The formation of the lithium amide base during the synthesis has been examined by ^{13}C MR and shown to be quantitative.

Additional quantities of several acyl- and diacylpyrazines have been synthesized and made available to Flavor Development for subjective evaluations:

CR-2043
CR-2044
CR-1945
CR-1946

Sodium borohydride reduction of the acylated pyrazines gives pyrazyl alcohols which may be considered as analogs of deoxyfructosazines. Preliminary results indicate that these alcohols partially dehydrate at 250° to give vinyl pyrazines.

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These compounds will be more thoroughly investigated as flavorants.

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PROJECT NUMBER: 2525
PROJECT TITLE: Tobacco Chemistry
PROJECT LEADER: S. Tafur
PERIOD COVERED: October, 1987

I. NATURAL PRODUCTS CHEMISTRY

- A. Objective: To isolate, identify, and/or analyze natural compounds with major emphasis on tobacco and tobacco products.
- B. Results: A standard curve was derived for the HPLC analysis of trans nicotine-N'-oxide (NN'O) in tobacco extracts. Estimates were made of the amounts of NN'O in extracts of tobacco leaves which had been fed unlabelled or ^{13}C -methyl-nicotine. Five additional types of tobaccos were analyzed and detectable levels of NN'O were found in a sample of Spanish Black tobacco and a moldy, partially cured, hydroponically-grown burley leaf. None was found by this method in samples of BL or supercritical CO_2 -extracted full flavor blended filler (1,2,3).

Fractionation of Bright tobacco extract fractions obtained from Flavor Development has begun. The purpose is to investigate non-volatile tobacco constituents as potential tobacco-natural flavorants for application to cigarettes. Initial reversed phase separation of Fr III has yielded a subfraction which appears to be concentrated in components whose chromatographic characteristics are similar to previously identified glucosides. Vacuum liquid chromatography (VLC) using C_{18} -silica in a funnel with a sintered glass frit has been shown to be a fast and efficient separation method which is capable of handling larger amounts of material than conventional preparative LC columns. Examination of other LC parameters found that aminopropylsilica was a good choice for resolution of fraction components. Separation of Fr IV on a preparative aminopropylsilica column (3.0 x 43.5cm) and a semi-preparative HPLC column (Zorbax NH_2) has produced two fractions highly concentrated in single, different compounds (4).

- C. Plans: Continue HPLC analyses of nicotine and its metabolites. Continue fractionation of Bright tobacco extract fractions and purification of specific compounds. Develop methods for subjective evaluation of fractions.

D. References:

1. Core, M. Notebook No. 8417, p.160-161.
2. Izac, R. Notebook No. 8379.
3. Izac, R.; Core, M. Preliminary analysis of ^{13}C -methyl-nicotine fed tobacco samples. Memo to J. Wooten, 1987 October 1.
4. Tafur, S. Notebook No. 8490, p. 93.

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II. GREENHOUSE STUDIES

A. Objective: To maintain the R&D greenhouses, to conduct plant research studies and to provide greenhouse-grown tobacco materials for support of other R&D programs.

B. Results: The harvest of roots from the sixth group of Burley 21 plants (35) grown in hydroponic culture produced 9.7 Kg of fresh root tissue for Project 1904. The seventh group of 36 plants has been transplanted to hydroponic tanks (1).

C. Samples of leaf tissue representing different areas within leaves were submitted for alkaloid analysis. The data will map the alkaloid distribution within leaves of greenhouse tobacco grown in hydroponic culture (1).

The plants in the York hydroponic culture experiment being fed with β -methyl valeric acid-2- ^{13}C have been topped (1). The flower and suckers are being freeze-dried (2,3).

Air-dried roots were harvested and weighed from tobacco plants grown in a culture x topping study. The root weight per plant was somewhat lower for the plants grown under dry culture than wet culture (102.3g/plant vs 126.8g/plant) (1).

C. Plans: Continue to produce fresh root tissue by hydroponic culture. Examine the sucrose ester production in the plants being fed β -methylvalerate K salt.

D. References:

1. Bass, R. Notebook No. 8495, pp. 76-81.
2. Izac, R. Notebook No. 8379.
3. Izac, R.R; Newman, R.H. Protocol for harvesting tobacco fed ^{13}C - β -methylvaleric acid. Memo to R. Bass, 1987 October 2.

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PROJECT NUMBER: 6505
PROJECT TITLE: Special Investigations/Methods Development
PROJECT LEADER: D. F. Ingraham
PERIOD COVERED: October, 1987

I. PESTICIDE LABORATORY

- A. Objective: Provide analytical data for pesticides in offshore leaf purchase.
- B. Results: The 25 samples which were in progress last month have been completed. No pesticides were out of limits. Eight additional samples received last month are still in progress.
- C. Plans: Continue analyses on the eight samples. Finish investigation on the new USDA herbicide method so that conclusions can be drawn as to its efficacy. Facilitate transfer of methods to QA.

II. PROJECT ART

- A. Objective: To investigate the role and fate of ammonia in the ammonium bicarbonate (AB) application.
- B. Results: AB determinations have been made on AB spray solutions and sprayed filler based on the determination of ammonia by an ammonia electrode. Hexane extracted nicotine values have also been determined for the sprayed filler to be used as a predictor of the carbon dioxide extraction efficiency of nicotine from the same filler. Equipment was installed in the pilot plant for the determination of AB containing materials and appropriate personnel have been trained on its use. It was determined that AB which had been hand-sprayed on filler did not lose ammonia under laboratory conditions during a 24 hour period. After one week there was a 64% decrease in soluble ammonia. Forty-nine additional nicotine analyses were completed and reported to Project ART personnel.
- C. Plans: To further investigate the loss of both ammonia and carbon dioxide during and after spraying.
- D. References:
 1. Davis, R. E., "Reporting AB Values," memo to R. Prasad, September 29, 1987.
 2. Davis, R. E., "Summary of AB Results for Park 500," memo to R. Prasad, September 30, 1987.
 3. Davis, R. E., "The Determination of AB on Filler," memo to R. Prasad, October 6, 1987.

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4. Davis, R. E., "Hexane Extractable Nicotine in AB Tobacco," memo to R. Prasad, October 12, 1987.
5. Davis, R. E., "AB Determinations in Filler and Solutions," memo to R. Prasad, October 12, 1987.
6. Davis, R. E., "Hexane Extractable Nicotine in AB Tobacco," memo to R. Prasad, October 15, 1987.
7. Davis, R. E., "Ab Determinations in Filler," memo to R. Prasad, October 15, 1987.

III. HEADSPACE ANALYSIS OF PACKAGING MATERIALS

- A. Objective: To provide headspace analyses of packaging materials for residual solvents.
- B. Results: A Hewlett-Packard headspace sampler was interfaced to an HP-5880A GC. Samples of packaging material and tipping paper were analyzed for volatiles. Analysis and reporting of results are currently in progress.
- C. Plans: To continue analyses as required.

IV. X-RAY FLUORESCENCE (XRF)

- A. Objective: To provide qualitative and quantitative elemental data on tobacco, cigarette paper and material evaluation samples.
- B. Results:

Qualitative Analysis

Materials evaluation: XRF was used to determine elemental content in GRAFOIL pipe sealant and AGELESS oxygen absorber. A foreign matter sample (FM-122), a brown dust from the floor of a transport trailer, was also analyzed.

Several paper, tobacco, and other samples were also analyzed.

Quantitative Analysis

Thirty-four samples were analyzed for 7 elements (Mg, Si, P, S, Cl, K, and Ca) using the Fundamental Parameters software.

- C. References:

1. Grantham, P. F., "Qualitative X-ray Fluorescence Scan of Smoke-Check Detector Strips," memo to R. A. Fenner, October 13, 1987.
2. PM Notebook #8543, pp. 48-49.

V. ATOMIC ABSORPTION

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- A. Objective: To provide elemental determinations on tobacco and cigarette paper samples.
- B. Results: A total of 296 atomic absorption determinations were performed this month. Sample types included treated filler, cigarette papers, modified size, CEL, SEL, and centrifuge sludges. A method for the determination of Se in ground filler has been developed. This method uses a modified microwave digestion of the sample and determination of Se using hydride generation atomic absorption. Thirty-four samples were analyzed using this method.
- C. Plans: Analyze thirty-one samples of Japanese cigarettes for Se.
- D. References:
1. PM Notebook #8394, p. 22.
 2. PM Notebook #8543, pp. 36-44 and 48-49.

VI. RESPONSE TO ANALYTICAL REQUESTS

- A. Objective: To provide analytical support to R&D and Operations personnel and projects.
- B. Results:
- Analyses and investigations by the project personnel during the month of October included:
- Nicotine determinations were performed on 246 samples from various submitters.
- Determination of methoxy content of several degraded pectin samples.
- Determination of ethanol content in several types of samples for the menthol on foil project.
- Two customer complaint samples were analyzed for possible contaminants.
- C. References:
1. Ingraham, D. F., "CC 870915001, Control no. 87095 - Marlboro Lights 100's - Clayton Iverson," memo to J. J. Pollard, October 2, 1987.
 2. Ingraham, D. F., "CC 870929034, Control no. 87097 - Merit KS - Beverly Allen," memo to J. J. Pollard, October 13, 1987.
 3. Ingraham, D. F., "Analysis of Red Pellet from Filter," memo to R. H. Cox, October 15, 1987.

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PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
PROJECT LEADER: J. M. Penn
WRITTEN BY: G. Nixon and J. M. Penn
PERIOD COVERED: October, 1987

I. INHIBITION OF EGF BINDING

- A. Objective: Establish the EGF assay and determine the effects of CSC on EGF binding.
- B. Results: An experiment was conducted this month to determine the quantitative properties of this assay by testing selected CSC samples. The cigarette samples tested were Kentucky Reference 2R1, the 100% bright expanded stems, and 75% DBC burley-25% bright expanded stems. The results indicated that all CSC samples exhibited equal growth inhibitory effects in a dose-dependent manner. Analysis of the binding data indicated that all CSCs inhibited EGF binding. Preliminary analysis suggests that there was no difference in activity between the CSCs tested, as the assay is currently being conducted. One interesting observation in this experiment was that the solvent control (DMSO) also significantly inhibited EGF binding. This finding could have profound effects on the degree of activity of the CSCs tested.
- C. Plans: Eliminate the effects of the solvent control on EGF binding.
- D. References:
- Stagg, D. L. Notebook 8553, pp. 49-64.

II. MAINTENANCE OF 3T3 CELL LINE

- A. Objective: Evaluate a new low passage 3T3 cell line in the EGF assay.
- B. Results: Because of extensive use of the routine 3T3 cell cultures (passage #224), it was decided to evaluate a new low passage 3T3 cell line. Both cell lines were tested in head-to-head experiments to determine normal EGF binding and the inhibitory effects of CSC on EGF binding. The results indicated that the new and old cell line produced similar values with regard to EGF binding and inhibition of binding with CSC. The early passage cells will now be routinely used in all future experiments.
- C. References:
- Patskan, G. J. Notebook No. 8527 pp. 87-134.

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III. GLUTATHIONE DEPLETION ASSAY (GDA)

- A. Objective: Evaluate the CSC activity from washed shredded bright stem (WSBS) cigarettes having whistle through (WT), charcoal, THAM on charcoal, and basic alumina filters.
- B. Results: The WSBS CSCs from cigarettes with WT, charcoal, THAM on charcoal, and basic alumina filters were tested to determine what effect filters (designed to remove aldehydes) would have on GDA activity.

Previous gas phase and whole smoke studies indicated that these filters reduced GDA activity. The results obtained from this experiment were surprising in that the CSCs from the charcoal and THAM on charcoal cigarettes were more active than the control WT CSC. The reason(s) for this finding is now under discussion.

- C. Plans: Test CSCs from cigarettes made with these filters and 100% DBC burley filler.
- D. References:

McCoy, W. R. Notebook No. 8555, pp. 16-17.

IV. ACYLARACHIDONYL GLYCEROL (AAG) IN 3T3 CELLS

- A. Objective: To isolate ^3H -AAG from 3T3 cells.
- B. Results: Thin layer chromatography of lipids extracted from cells grown on permanox (plastic) plates did not show a region of radioactivity corresponding to AAG. Re-chromatographing the sample in a different solvent system indicated that the majority of the ^3H was incorporated into phospholipids. A second experiment using cells grown on glass vs. permanox still did not show ^3H -AAG on TLC (1). In a third experiment, the amount of sample spotted on TLC was greatly increased, and preliminary indications are that concentration of the sample is a factor, because there was radioactivity detected in the region corresponding to AAG (2).
- C. Plans: Develop experimental procedures to concentrate and quantitate lipid extracts from 3T3 cells.
- D. References:

Nixon, G. Notebook No. 7758, pp. 165-168, 173-176.
Nixon, G. Notebook No. 8569, pp. 1-4, 7-8.

V. SALMONELLA/MICROSOME (S/M) ASSAY

- A. Objective: To determine if methyl glyoxal could be used as a positive control for TA104.

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- B. Results: In spite of low titres and high background from experiments with TA104, the strain responded favorably to titres, sensitivity checks, 2R1 CSC and methyl glyoxal as a positive control.
- C. Plans: Reisolate TA104 strain and evaluate IT CSC's from 7 model CSC cigarettes.
- D. References:

Coleman, S. L. Notebook No. 8465, pp. 91-93.

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PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: R. D. Kinser
WRITTEN BY: R. J. Levins
PERIOD COVERED: October, 1987

I. TSNA PRECURSORS

- A. Objective: To determine the precursors of MS TSNA.
- B. Results: Spray application of a protein (1% level), bovine serum albumin (BSA), to oriental base web had no effect on smoke NO or TSNA delivery. Addition to burley base web at 2% actually reduced the delivery of all TSNA by about 30%.

Attempts to confirm the identity of the component identified by GC retention time and TEA response as NNK by preparation of the 2,4-dinitrophenylhydrazine derivative have been unsuccessful. Under the acidic conditions required, NNK and similar model compounds form salts which do not chromatograph via GC or LC. Other approaches will be investigated.

TSNA analyses were performed on partially air-cured (7 weeks) hydroponically grown bottom leaves from two Burley 21 plants. Although the TSNA amounts are not up to normal fully cured burley levels, TSNA are detected at levels of 3-14% relative to normal DBC burley. The fully cured leaves will be analyzed when available.

DBC burley (J7AAM) was exhaustively water-washed to prepare a substrate for salt add-back studies. The resulting filler contained only traces of TSNA.

The absolute minimum detection levels for TSNA by GC/TEA were established via serial dilution of the calibration standard. The limits obtained are: NNN = 28 pg; NAT = 41 pg; and NNK = 103 pg.

C. Plans:

- Replicate protein (BSA) addition to base web
- Confirm identity of NNK isolate
- TSNA analysis of fully cured, hydroponically grown Burley 21 leaves

D. References:

Haut, S. A. Notebook No. 8468, p. 159.
Morgan, W. R. Notebook No. 8506, p. 185.

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II. TSNA REMOVAL STUDIES

- A. Objective: To explore the possibility of extracting TSNA from stored tobacco using an extraction fluid compatible with current processing.
- B. Results: Work continued on the development of a convenient, easily maintained HPLC method for determining nicotine and important minor alkaloid in filler. Increasing the length of the cyano column from 15 to 25 cm permits the additional measurement of anatabine, anabasine and nornicotine in the HPLC method for nicotine in filler. Various solvent systems are being investigated to improve filler extraction without interfering with the chromatography. C-18 SPE cartridges are being used to concentrate filler extracts which contain low levels of the minor alkaloids (e.g., from previously extracted tobaccos). At pH 11, the alkaloids are retained; salts are removed with water, and the alkaloids are then eluted with the HPLC solvent.
- C. Plans: Find a filler extraction solvent that yields adequate alkaloid recoveries and that is compatible with HPLC requirements.
- D. References:
Warfield, A. H. Notebook No. 8558, p. 8.

III. DECOMPOSITION OF TSNA

- A. Objective: To evaluate the thermal stability of TSNA and investigate methods to enhance thermally induced decomposition of these compounds.
- B. Results: Studies of TSNA decomposition in the presence of the antioxidant ascorbyl palmitate (AP) continued. At 300°, 250° and 200°C, NNN decomposition values agreed well with previously obtained values. Values for NNK, however, were not consistent with earlier data. Both the NNN and NNK values were obtained using the same experimental set-up.
- C. Plans: The NNK experiment will be repeated.
- D. Reference:
Morgan, W. R. Notebook No. 8506, p. 185.

IV. ALTERNATIVES TO CARBON TRAPPING OF NICOTINE IN SCFE TOBACCO

- A. Objective: To determine the efficacy of other nicotine trapping systems in the removal of TSNA from the process stream.

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- B. Results: Analysis of a new stem material being used as the nicotine adsorber in the supercritical fluid extraction of tobacco and analysis of the tobacco indicates that TSNA levels are reduced in the extracted filler.
- C. Plans: Analyses will continue to be carried out as required.
- D. References: Haut, S. A. Notebook No. 8468, p. 159.

V. CARBONYL CONTENT OF SMOKE FROM A MODEL CIGARETTE OF LOW BIOLOGICAL ACTIVITY

- A. Objective: To determine the chemical components responsible for the poor subjective of smoke from a model cigarette of low biological activity (washed shredded bright stems), and to decrease the levels of these components.
- B. Results: Last month it was reported that the WSBS cigarette X6D7CEA (whistle-through filter; control) gave an unusually high combined acrolein/acetone peak (1824 ug/cig.), which appeared to be drastically reduced by a THAM (trishydroxymethylaminomethane) P/S/P filter. The control cigarette was re-smoked and analyzed using both the usual acetonitrile/water HPLC gradient, which gives a combined acrolein/acetone peak, and a newly developed methanol/water gradient, which clearly separates these peaks. The values obtained (466 vs. 201 + 255 ug/cig., respectively) are in nominal agreement with other WSBS models. It is quite probable that the earlier high value was an artifact produced when the X6D7CEA control was the first cigarette smoked after the rotating head of the Borgwaldt smoking machine had recently been cleaned with acetone.

Three models of WSBS and two models of FFB cigarettes (with and without THAM filters) were sent to H. Spielberg for limited subjective panel testing.

- C. Plans: Continue evaluation of various model systems as required.
- D. References:

Levins, R. J. Notebook No. 8558, p. 47.

V. CROSSED SOLUBLES/BASEWEB STUDY (CHEMISTRY)

- A. Objective: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley, and oriental tobaccos on base webs from the same three tobaccos.
- B. Results: The dialysis performed last month on CEL sub-fraction BuS1 gave a recovery of less than 13% of the exchanged material. This is very surprising in that a 1,000MW cut-off membrane bag was employed. This work will be continued based on the encouraging

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drop in total salts: BuS1 (control) - 29.5% salt; BuS1 (dialyzed) - 2.50% salt.

The North Panel has begun subjective evaluation of the crossed solubles/BW cigarettes.

NRM (ninhydrin reactive material) and protein values were determined on 26 samples obtained from three different experiments which utilized reducing sugars (glucose and fructose).

C. Plans: Continue to modify CEL fractions for RL preparation.

D. References:

Williams, D. Notebook No. 8530, p. 22.

VI. SUPPORT FUNCTION; CONDENSATE PREPARATION

A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.

B. Results: The CSC from four specially prepared models of WSBS cigarettes with modified filters was collected and furnished as DMSO solutions for a glutathione depletion assay time study.

C. References:

Hellams, R. D. Notebook No. 8480, p. 177.

Lambert, E. A. Notebook No. 8523, p. 49.

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PROJECT NUMBER: 8101
PROJECT TITLE: Cigarette Testing Services Division
SECTION LEADER: Joyce F. Stargardt
PERIOD COVERED: October, 1987

I. MARKET ACTIVITY

A. Objective: To monitor the introduction of new brands and brand modifications to existing brands and to report the domestic market activity on a monthly basis.

B. Results:

1. New Brands

The House of Sampoerna, an Indonesian cigarette manufacturer, is distributing three clove cigarettes in California: 234 90 mm (Box), 234 Filter Slims 100's (Box), and X-tra 90 mm (Box). The X-tra cigarettes have black cigarette paper and black tipping paper. Sweetened tipping paper is used on the 234 Filter Slims and the X-tra cigarettes. The 234 cigarettes deliver 42 mg tar and 1.9 mg nicotine; the Filter Slims deliver 32 mg tar and 1.5 mg nicotine; and the X-tra cigarettes deliver 45 mg tar and 1.6 mg nicotine.

2. Brand Modifications

R. J. Reynolds has increased the ventilation (63 to 73%) of Now 100 cigarettes resulting in lower tar (3.4 to 2.5 mg), nicotine (0.29 to 0.21 mg), and CO (4.3 to 2.4 mg) deliveries.

Lorillard has decreased the ventilation (57 to 48%) of Kent III 100 cigarettes resulting in increased tar (4.7 to 6.1 mg), nicotine (0.46 to 0.54 mg), and CO (4.9 to 7.4 mg) deliveries.

3. Unidentified 100 mm Cigarette

R. J. Reynolds is panel testing an unidentified 100 mm product with magnesium oxide paper. This product has a 10 mg tar delivery and a mechanically perforated cigarette paper. It is low in both visibility and mass burn rate. The filter contains a "red pellet" which is a co-polymer of ethylene and vinyl acetate. No flavoring or other additives were identified in this pellet.

II. ANALYTICAL METHODS DEVELOPMENT AND SUPPORT

A. Objective: To evaluate and recommend analytical methods and new technology in support of programs for R&D and Manufacturing.

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B. Results:**1. Freon®**

Reynolds, American, Liggett and Lorillard continue to use Freon® expanded tobacco in their products. Freon® was not detected in the filler of Philip Morris or Brown and Williamson brands.

2. Menthyl Glucosyl Carbonate (MGC)

Menthyl Glucosyl Carbonate is a menthol release compound under evaluation for a mentholated charcoal cigarette. Menthol is released from this compound upon pyrolysis and/or hydrolysis. There are several isomers each having different menthol release efficiency. At the request of G. Yatrakis, seven solutions of MGC in ethanol were analyzed for menthol by standard gas chromatographic (packed column) procedures. The premise was that the menthol would be released in the heated injection port of the gas chromatograph, and the glass injection port liner would provide a chamber for the reaction. The solutions were analyzed on three Varian 3700 gas chromatographs (equipped with glass lined injection ports) over a five day period. The experimental results were compared to the theoretical menthol targets (50 - 200 mg menthol/mL) based on the menthol release efficiency for the particular batch of MGC. A standard menthol aftercut solution was analyzed as a control.

Experimental menthol concentrations averaged 14% below target for the MGC solutions. The control averaged only 4% above target. The major source of error was attributed to the inability to control the retention time of the sample in the injection port using standard packed column gas chromatographs which significantly impacted the menthol release. In addition, injection port cleanliness and injection speed also affect the efficiency of pyrolysis and hydrolysis of this compound. Based on these results, menthol released by MGC in a heated injection port can not be accurately measured using standard packed column gas chromatography.

3. Robotics

The second smoking robot was installed and is now being used routinely to generate TPM and puff count data. Installation of the third smoking cubicle robot began in October. Plans are to have this robot operational by the second week of November.

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